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## Progress Report for the Dairy Research Advisory Board

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Dairy Research Advisory Board  
Eccles Conference Center 201-203  
Utah State University

July 25, 1984

AGENDA

- 8:30 AM Welcome. Dr. D.J. Matthews, Director, Utah State University Agricultural Experiment Station
- 8:40 AM PROJECT REVIEWS
1. Calcium and the meltability of process cheese food made from ultrafiltered milk.  
C.A. Ernstrom
  2. Evaluation and improvement of coagulation properties of milk for manufacture.  
Leslie M. Okigbo
  3. Effect of whey protein denaturation in dried whey used for bread making.  
Ronald Malouf
  4. Production of a high moisture white cheese from ultrafiltered milk.  
S.M.K. Anis
  5. Role of milk clotting enzymes in cheese curing.  
C.A. Ernstrom
  6. Cottage cheese from ultrafiltered skim milk.  
Ronald M. Raynes
- Lunch. Center Colony Room - Taggart Student Center.
7. Plasmid profiles of proteinase positive and negative strains of S. cremoris.  
Craig J. Oberg
  8. Casein/fat ratio and fat recovery in Cheddar cheese.  
Nana Yiadom-Farkye

9. Progress with proteinase negative culture research and measurement of milk coagulation properties in cheese vats.

Steve Winkel and Gary H. Richardson

10. Proteinase negative cultures in Cheddar cheese manufacture.

Craig J. Oberg

11. Estimation of milk protein concentration by amino acid analysis.

Rodney J. Brown

12. Effect of exercise and cheese supplemented diets on levels of cholesterol and lipoprotein fractions in human subjects.

Kathe A. Gabel-Lind

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Utah State University  
July 28, 1983

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11. Chr. Hansen's Laboratory Inc.  
Dr. Robert Sellars  
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12. Clearfield Cheese Co.  
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Curwensville, PA 16833  
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13. Middlefield Cheese  
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16. General Mills, Inc.  
James Ford Bell Technical Center  
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Minneapolis, MN 55427  
Dr. S.F. Sapakie  
Dr. J. Efsthathiou

# Calcium and the Meltability of Process Cheese Food Made From Ultrafiltered Retentate

S.M.K. Anis and C.A. Ernstrom

Some of the information in this report was presented in last years report. However, it was felt that it should be repeated in conjunction with additional experiments that bear on the problem of meltability of process cheese products.

Work by Dr. Paul Savello on a model system of casein and milk fat plus controlled addition of other ingredients was designed to determine the cause of poor meltability of process cheese food made from ultrafiltered whole milk retentate.

Results from the model system revealed that high whey protein concentration, high calcium concentration and poorly hydrated casein (particularly acid casein) all contributed to poor melting characteristics in process cheese food.

Cheese curd made from ultrafiltered whole milk retentate subsequently reduced to 38% moisture contains about 4.0% whey proteins. Traditional Cheddar cheese would be expected to contain about .5%. A major part of the improved yield associated with UF cheese curd for processing results from whey protein recovery. Therefore, the economic attractiveness of the process would be lost if whey protein recovery were reduced. Hydration of the casein in curd made from ultrafiltered whole milk does not present the same problem found during reconstitution of dry casein such as was used in our model system. This leaves the control of effective calcium concentration as the most likely way to control melting properties in process cheese products made from ultrafiltered whole milk.

This study was conducted to show the relationship between calcium removal from milk during ultrafiltration and meltability of process cheese food made from the U.F. curd. The effect of various emulsifying salts and calcium salts added during cheese processing also will be reported.

Ultrafiltration of pasteurized whole milk was at 54°C with a 5M<sup>2</sup> Abcor Spiral wound polysulfone membrane with a nominal molecular weight cut off of 5000. Operating pressures were 60 psi on the inlet and 40 psi on the outlet.

Cheese curd was prepared by ultrafiltering whole milk to a 2.5X concentration followed by constant volume diafiltration with deionized water, then final ultrafiltration to 5X concentration. The final retentate containing 60% moisture and 20% fat was inoculated with 1.0% lactic culture at 30°C and fermented to pH 5.1.

A scraped-surface vacuum evaporator operating at 50°C and 23 inches of vacuum was used to reduce the moisture of the fermented retentate to 38%.

The UF cheese curd was processed in a 3 kg batch cooker at 73-75°C for 1 minute. Emulsifying salt, NaCl, and water were added. The final product contained 2.5% emulsifying salt, 4.5% salt in the cheese moisture and 42.5 - 43.5% moisture.

Meltability of the process cheese food was measured in duplicate by a modification of the method of Olson and Price. Samples were heated simultaneously in a forced air oven at 110°C for 1 hour and meltability measured in millimeters of flow.

Calcium was determined by ashing the samples at 550°C, dissolving the ash in 6M HCl, diluting to appropriate concentrations and measuring by Atomic Absorption Spectrophotometry.



Calcium concentration in the final cheese curd was varied by cold acidification of the milk to pH 6.6, 6.4, 6.2, 6.0 and 5.8 with concentrated hydrochloric acid prior to ultrafiltration. The amount of diafiltration water used varied from 38.5% of the original milk weight for milk at pH 6.6 to 70% for milk at pH 5.8.

Slide 1 illustrates the percent of original milk calcium removed at all stages during the ultrafiltration-diafiltration process. The beginning and end of diafiltration is marked by arrows on the respective curves. The upper curve represents UF of milk at pH 5.8. The lower curve represents UF of milk at pH 6.6. Calcium concentrations in the final retentate varied from .31% at pH 6.6 to .22% at pH 5.8. Loss of calcium during ultrafiltration resulted not only from solubilization of colloidal calcium phosphate by acidification, but also by more extensive diafiltration employed with each decreasing increment of pH.

The effect of acidification of milk and calcium removed on the meltability of the process cheese food made from the curd is shown in slide 2. Meltability is expressed in terms of millimeters of flow during the melting test. It is apparent that a substantial improvement in melting quality was realized by acidification of the milk prior to ultrafiltration. However, ultrafiltration of acidified milk has one serious drawback in that it substantially retards the flux rate. Slide 3 shows the changes in flux rate experienced during the ultrafiltration-diafiltration process of the milk. Acidification of milk to pH 5.8 resulted in curd which produced the best melting process cheese food but had an initial flux rate less than half that of milk at pH 6.6.

Milk acidified to pH 5.8 was next ultrafiltered and made into curd for processing. The effect of five different emulsifying salts on

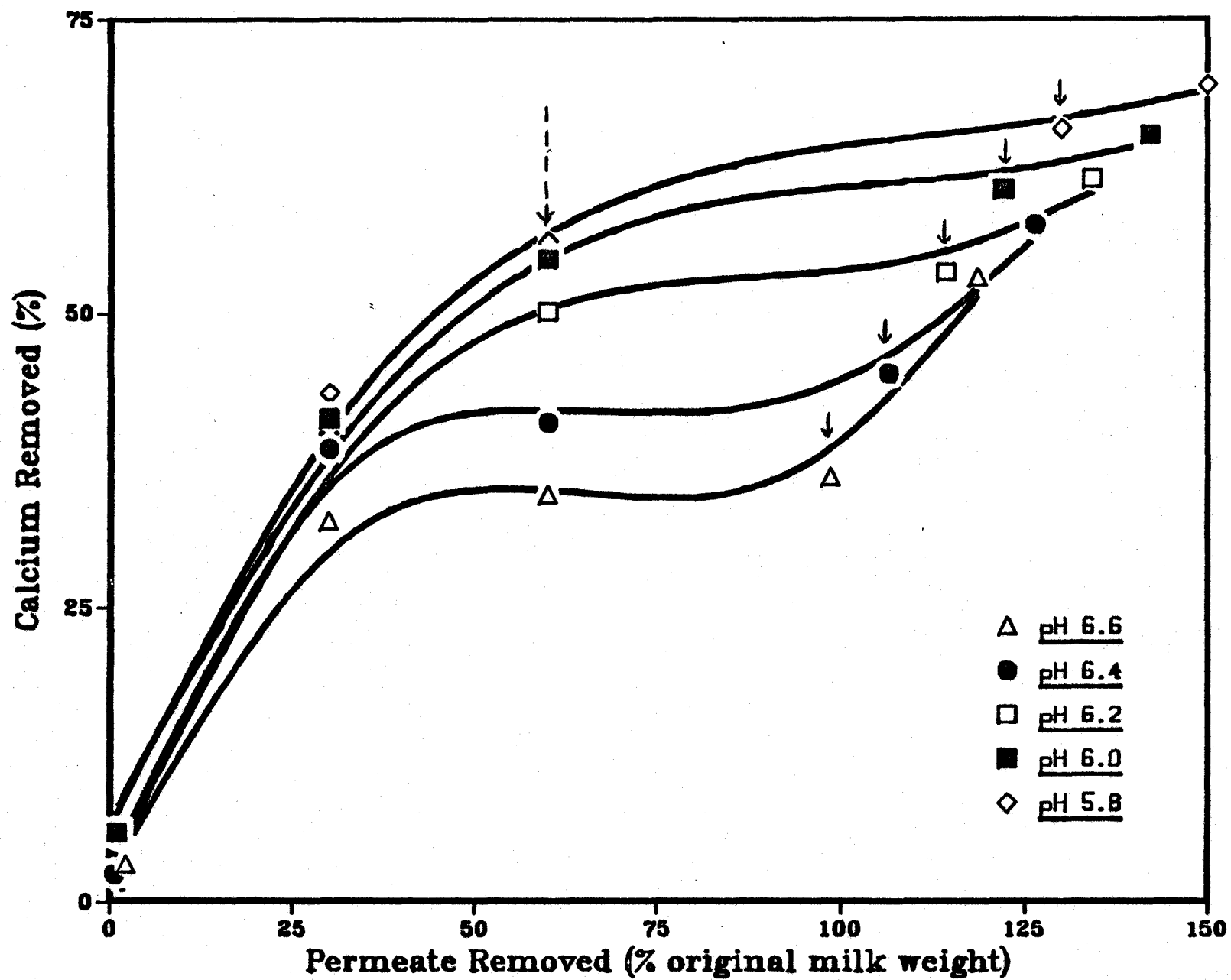


FIG 1

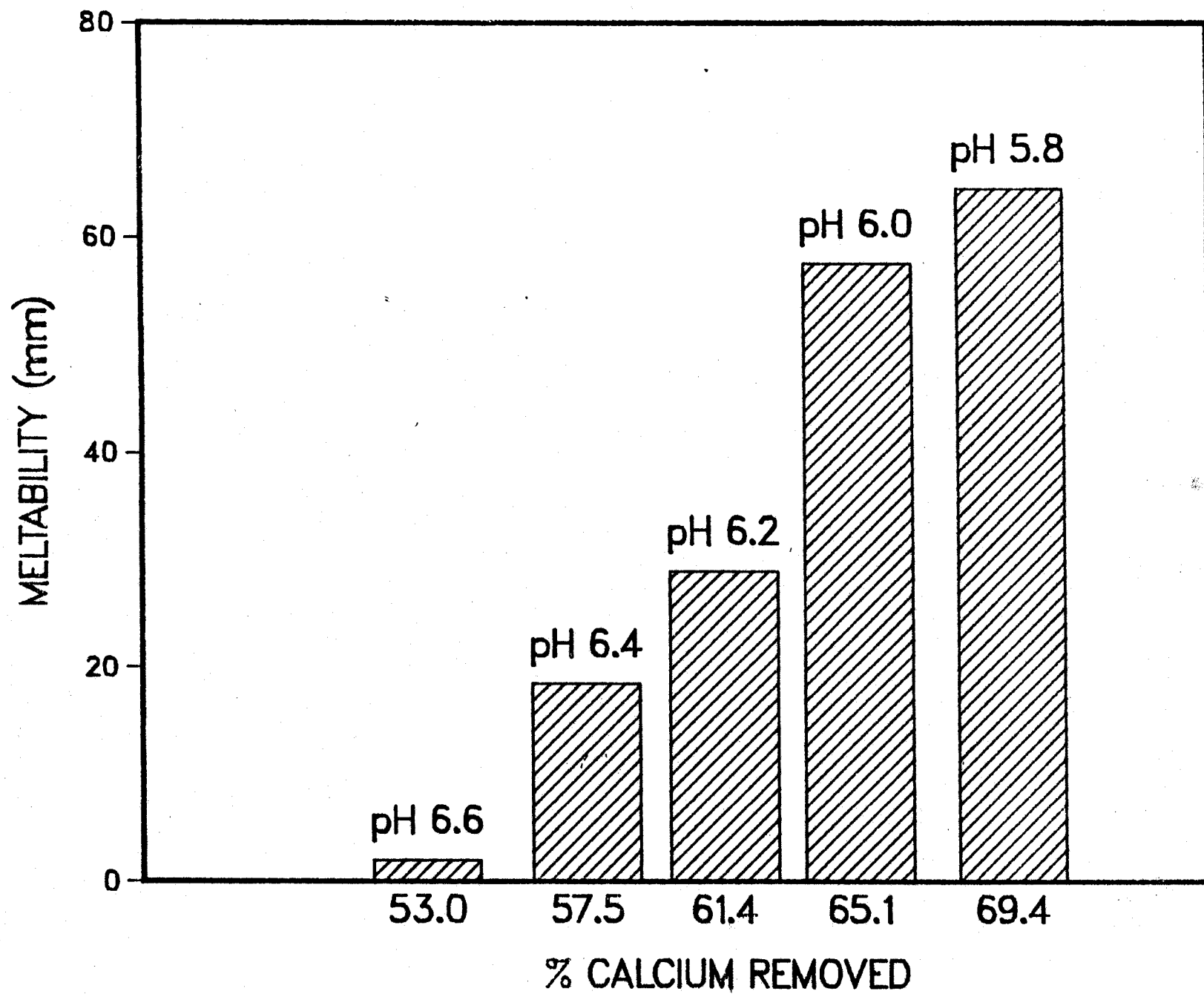


Figure 2

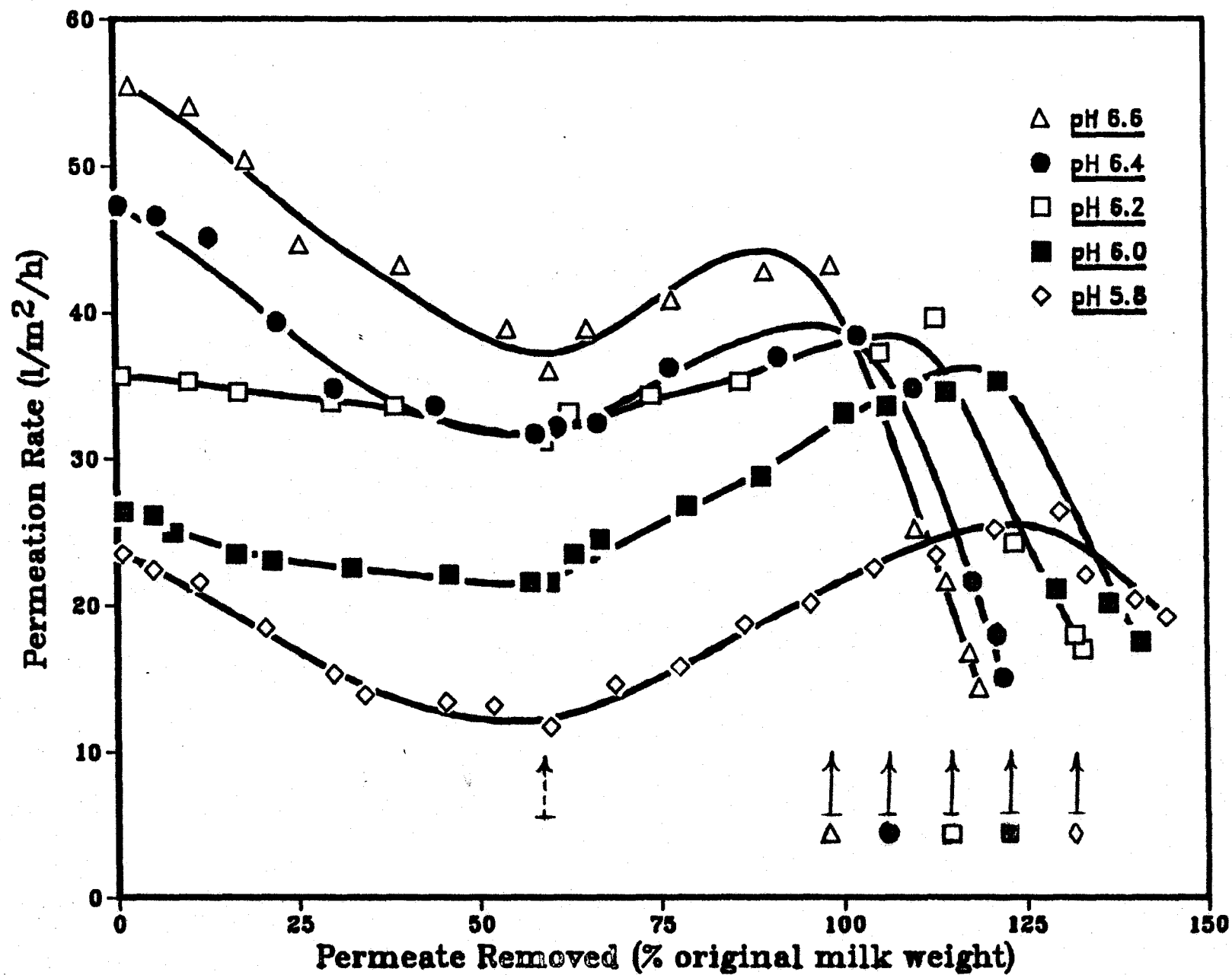


Figure 3

meltability of pasteurized process cheese food was evaluated and is shown in slide 4. Sodium citrate and sodium aluminum phosphate were the only ones that resulted in products with acceptable melting properties.

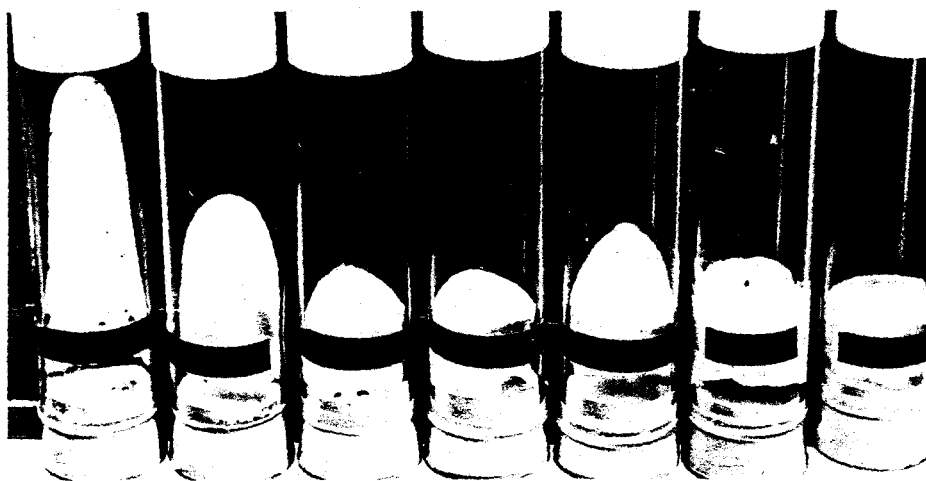
Curd made from milk ultrafiltered at pH 5.8 contained only about .4% calcium, which is less than that normally found in fresh Cheddar cheese curd. Fortification of the curd to a level of .6% calcium with various salts at the time of processing detracted from the melting properties of the cheese (slide 5).

The effect of the added calcium on meltability was overcome by increasing the sodium citrate emulsifier to 5.0%. The effect of the amount sodium citrate is further illustrated in figure 6 where the curd was fortified to .6% calcium with calcium citrate and processed with three different levels of sodium citrate.

The meltability of processed cheese food made from ultrafiltered whole milk is particularly sensitive to the calcium content of the curd. Meltability can be substantially improved by acidification of the milk prior to ultrafiltration in order to solubilize and remove calcium. This procedure will, however, be at the expense of a lower UF flux rate.

Even though calcium was reduced through acidification of milk before ultrafiltration, satisfactory meltability was achieved only when sodium citrate, and to some extent sodium aluminum phosphate were used as emulsifying salts. Attempts to fortify curd with several calcium salts reduced the melting properties of the processed cheese food. These were restored only by increasing the level of sodium citrate in the cheese cooker.

Treatment of the milk retentate with rennet at any time before or during fermentation destroyed the melting properties of process cheese



2.5%  
Sodium  
Citrate

Control  
(No Ca.  
added)

Ca.  
Ci-  
trate

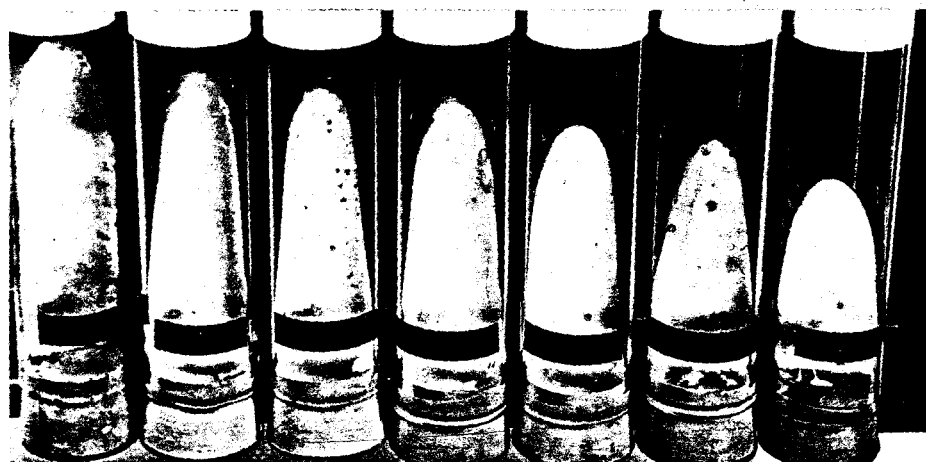
Ca  
Phos-  
phate  
(dibasic)

Ca.  
Chlo-  
ride

Ca.  
Lactate

Ca.  
Carbo-  
nate

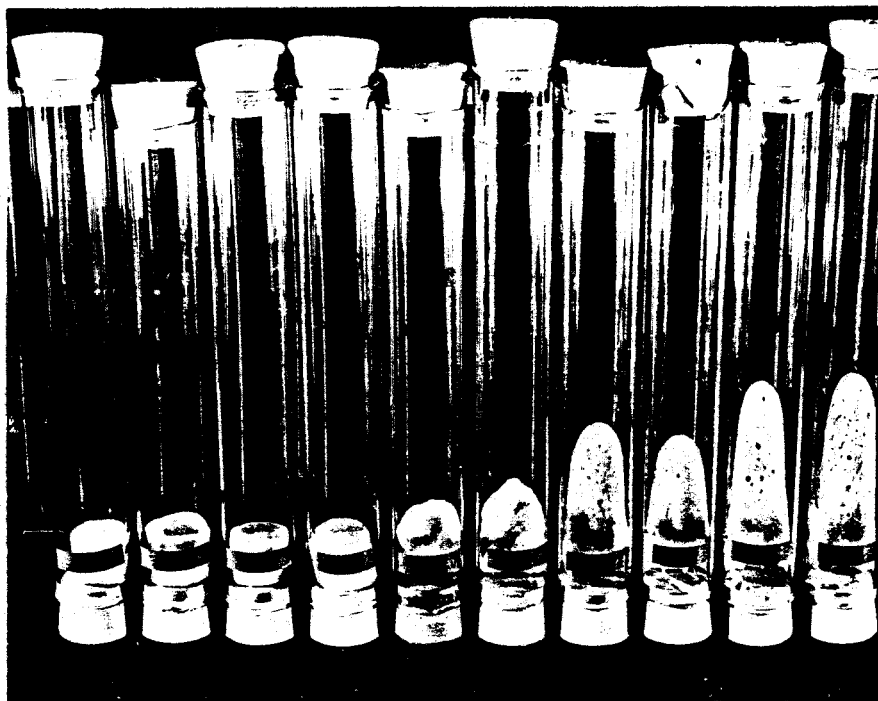
Ca.  
Gluco-  
nate



5.0%  
Sodium  
Citrate

Meltability of process cheese food made from acidified whole milk retentate fortified with calcium salts to raise the calcium from .4 to .6%.

Figure 4



Meltability of process cheese food made from UF acidified milk (pH 5.8) using different emulsifying salts.

A = Tetrasodium phosphate	B = Trisodium phosphate
C = Disodium phosphate	D = Sodium aluminium phosphate
E = Sodium Citrate	

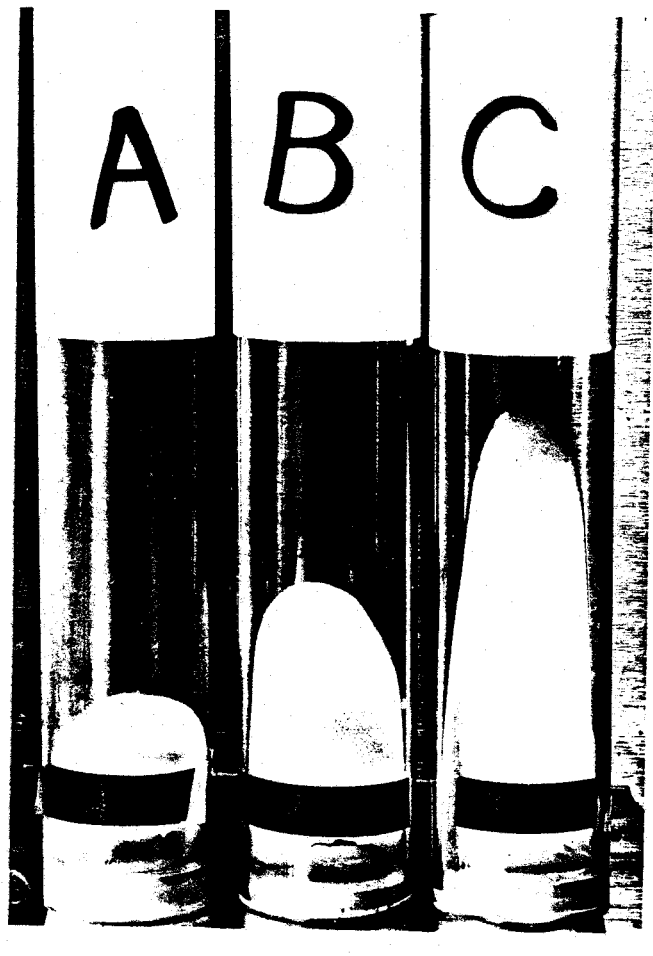
*Figure 5*

% Sodium Citrate

1.8

2.5

5.0



Meltability of process cheese food made from acidified UF milk fortified with Calcium citrate to raise the calcium level from .4 to .6% and emulsified with three levels of sodium citrate.

*Figure 6*



presumably because of its effect on increasing the sensitivity of the caseinate system to calcium.

EVALUATION AND IMPROVEMENT OF COAGULATION PROPERTIES  
OF MILK FOR MANUFACTURING

- I. Effects of pH, calcium chloride, and chymosin concentration on coagulation properties of abnormal and normal milk.

by

Leslie M. Okigbo

## INTRODUCTION

Inability of milk from some individual cows to coagulate, especially in late lactation, has been reported (21). This phenomenon seemed more apparent in fall and winter months in the Northern hemisphere. The obvious impact is a loss of cheese yield resulting from non convertibility of milk solids (casein plus fat) into cheese. Addition of .02% (about 1.8 mM)  $\text{CaCl}_2$  to cheese milk, especially during winter, improves coagulation and produces about 32% increase in curd firmness (12). Even though  $\text{CaCl}_2$  produces firmer curd, other factors also may improve coagulation properties of poor chymosin-coagulating milk (PCM). Curd firmness may increase up to 81% by addition of about 10 mM  $\text{CaCl}_2$ , but higher levels cause a decrease (12,20). Such high levels are not practical at present in commercial operations because of legal limits (8). High levels of calcium also have been associated with curd meltability problems in processed cheese (23). Other modifications that would produce higher curd firmness without excessive use of  $\text{CaCl}_2$  are desired.

This work summarizes the effects of variation of the levels of some factors that affect milk coagulation on coagulation properties of milk which exhibit PCM characteristics.

## METHODS AND PROCEDURES

### Selection of milk samples

Fifty milk samples from individual cows were obtained from Utah State University Holstein herd in December 1982 at one sampling. Cows were selected to cover all stages of lactation. Samples were stored overnight at 4°C to guarantee uniform temperature history. The pH of milk samples was measured at 37°C with a Beckman Model 60 pH meter (Beckman Inc., Fullerton, CA). Ten milliliters of each sample was then coagulated with 200 µL of diluted chymosin (.4 rennin units (RU)/mL) after tempering at 37°C for 90 min. Coagulation properties of the samples were recorded with a Formagraph instrument (18). Fifteen good chymosin-coagulating milk (GCM) and 15 PCM samples were selected based on their curd firmness and the source cows identified.

Fifty percent blends of selected individual GCM and PCM were prepared by adding 5 mL of one GCM to 5 mL of one PCM. This gave a total of 15 blends. The pH's of the blends were measured after tempering at 37°C for 90 min. They were then coagulated in the Formagraph.

Two milliliters of individual GCM samples were pooled together and the PCM samples were similarly pooled. Fifty percent of the pooled GCM was mixed with 50% of the pooled PCM and the final pH of each pool was measured after tempering at 37°C for min. Berridge substrate (4) and 12% low heat, spray dried, reconstituted nonfat dry milk (RNDM)

controls were tempered at 37°C for 90 min and were then compared with the pooled samples for chymosin-coagulation properties.

#### Modification of milk samples

The pH of each 10 mL aliquot of individual GCM and PCM samples was reduced to 6.3 by adding 1 to 3 drops of 2.1 N lactic acid solution during constant stirring at 37°C. The samples were left to equilibrate at 4°C for 24 h then minor shifts in pH were adjusted with lactic acid at 37°C. The samples were tempered in a water bath at 37°C for 90 min then coagulated in a Formagraph. Modification of pH was necessary because previous work on variations in coagulation properties of milk from individual cows (21) showed that pH change was a significant factor in altering coagulation properties. In this study, preliminary observation with Berridge substrate on the effect of pH on coagulation time (Figure 3) showed that coagulation time was prolonged at pH 6.7. Optimum curd firmness was obtained at pH 6.2, 30 min after chymosin addition (Figure 4) at the concentration of chymosin used (.4 RU/mL).

Ten milliliters aliquots of individual GCM and PCM samples were adjusted to pH 6.3 and .02%  $\text{CaCl}_2$  was added. The samples were tempered at 37°C for 90 min during which time minor shifts in pH were readjusted at 30 min intervals. The samples were then coagulated in the Formagraph.

The effect of varying chymosin concentration on coagulation properties was investigated. In a preliminary

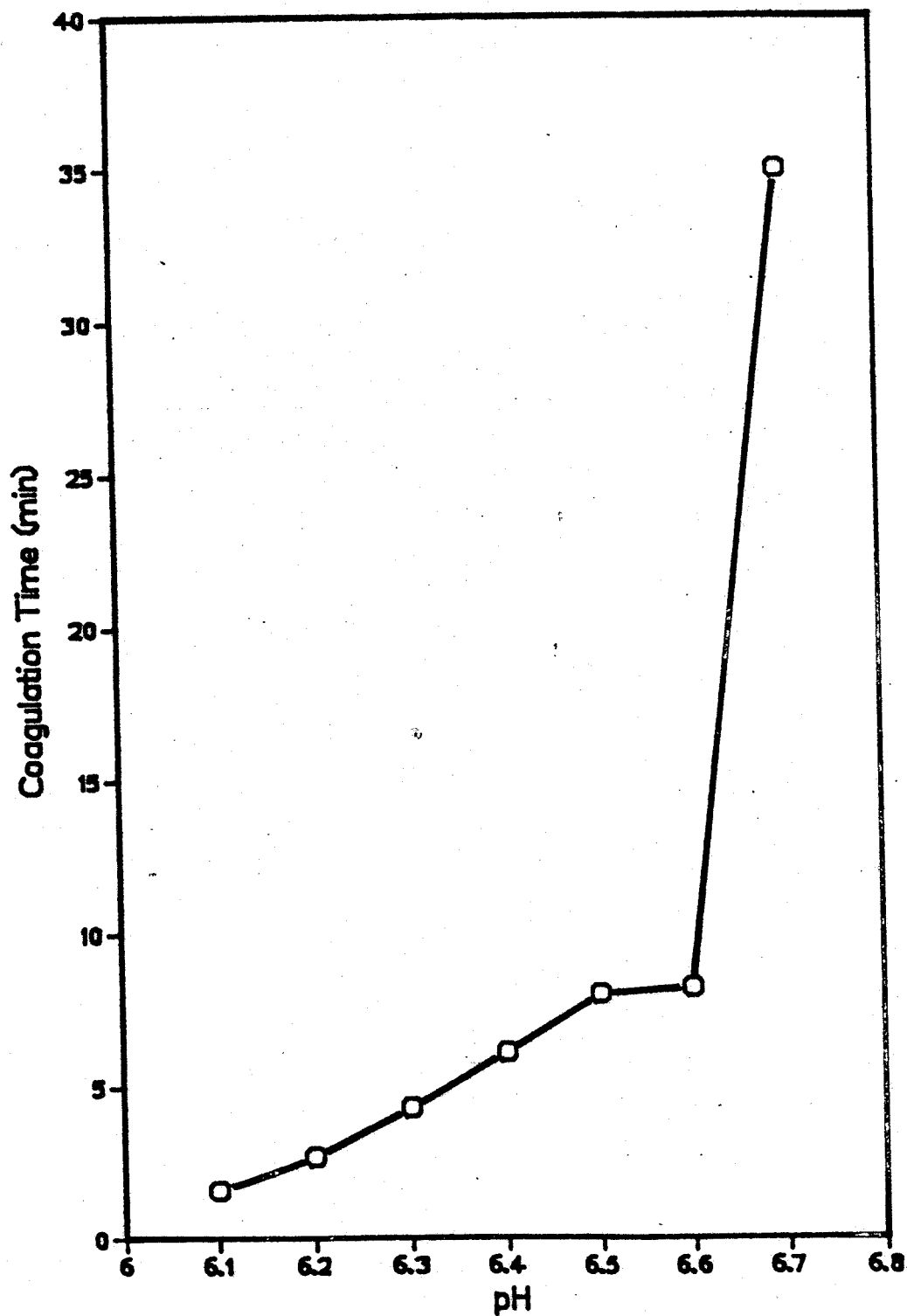


Figure 3. Effect of pH on chymosin coagulation time of Berridge substrate at pH 6.3

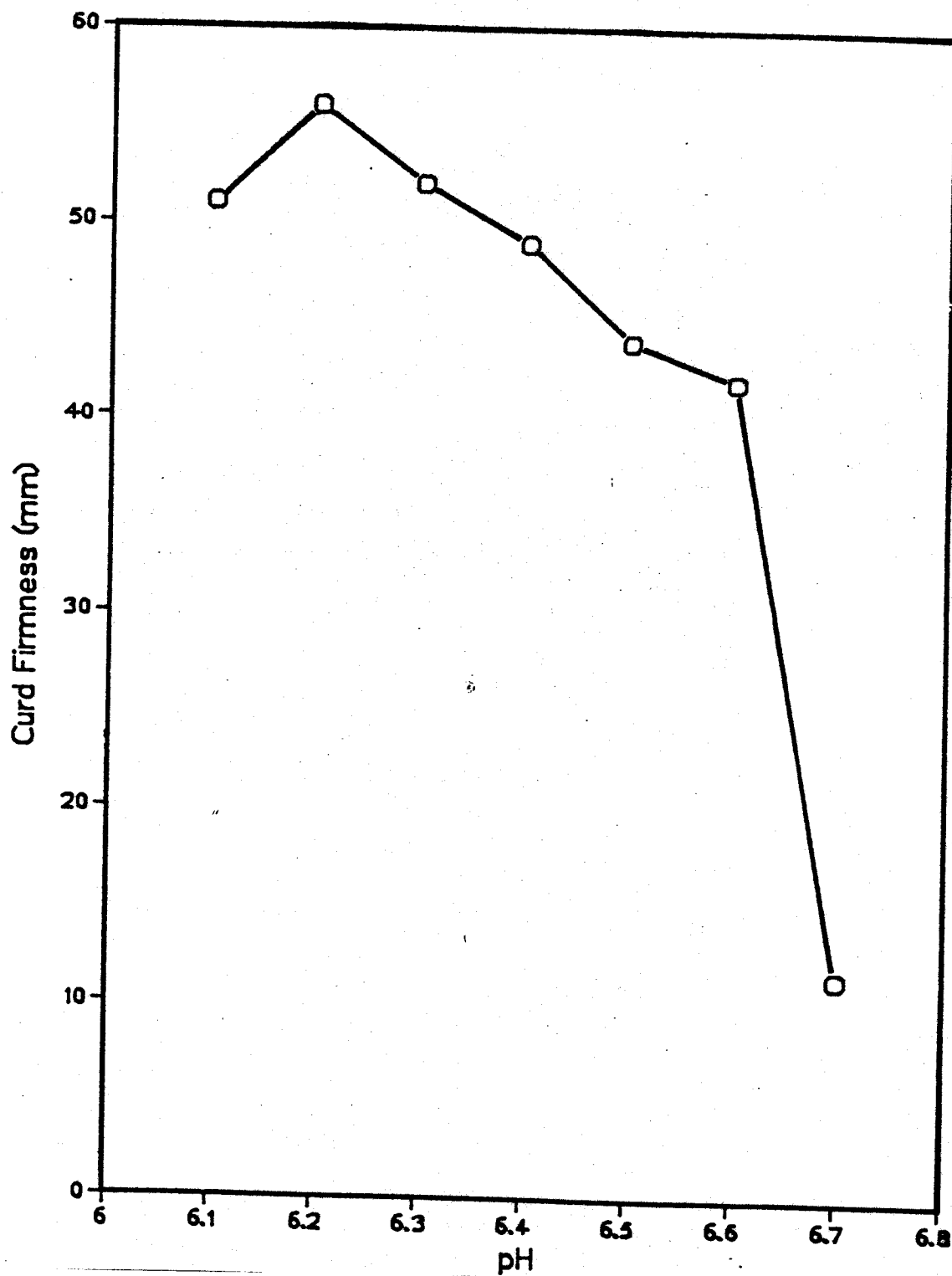


Figure 4. Effect of pH on curd firmness of Berridge substrate 30 min after chymosin addition.

study with Berridge substrate at pH 6.3, low chymosin concentration (.2 RU/mL) produced curd firmness 30 min after chymosin addition which was not significantly different from curd firmness produced by .3 to .5 RU/mL.

Chymosin concentration was then reduced from .4 to .2 RU/mL (18), the pH of each GCM and PCM sample was reduced to 6.3 from their natural pH, then minor shifts in pH readjusted while tempering at 37°C for 90 min.  $\text{CaCl}_2$  (.02%) was added before tempering. The milk samples were then coagulated in the Formagraph.



## RESULTS AND DISCUSSION

### Distribution of individual samples according to their coagulation properties

The distribution of 50 individual cow milk samples according to their coagulation properties is shown in Figures 5 and 6. The mean coagulation time for all samples which coagulated was 10.6 min, 15.9 min for 12% RNDM and 4.3 min for Berridge substrate. Jen and Ashworth (12) similarly observed a shorter coagulation time with fresh whole milk than RNDM. The distribution of the milk samples according to curd firmness showed 68% of the samples satisfactory (31 to 53 mm). The other samples had relatively weak curds (less than 30 mm). Mean curd firmness 30 min after chymosin addition for all the samples was 32.4 mm, 33 mm for 12% RNDM and 55 mm for Berridge substrate.

The 15 GCM and the 15 PCM samples selected on the basis of curd firmness are shown in Table 1 with corresponding pH values. Curd firmness tended to decrease as pH increased, but samples with pH greater than 6.85 generally did not coagulate. This observation is in agreement with previous findings (7,15,21) which indicate that pH change is highly significant in altering the firmness of milk curd. Changes in pH are known to affect enzyme activity (9,22). This is also illustrated in Figures 5 and 6.

### Effect of blending individual samples

The effect of coagulating a blend of GCM and PCM is illustrated in Table 1. The PCM reduced the overall curd

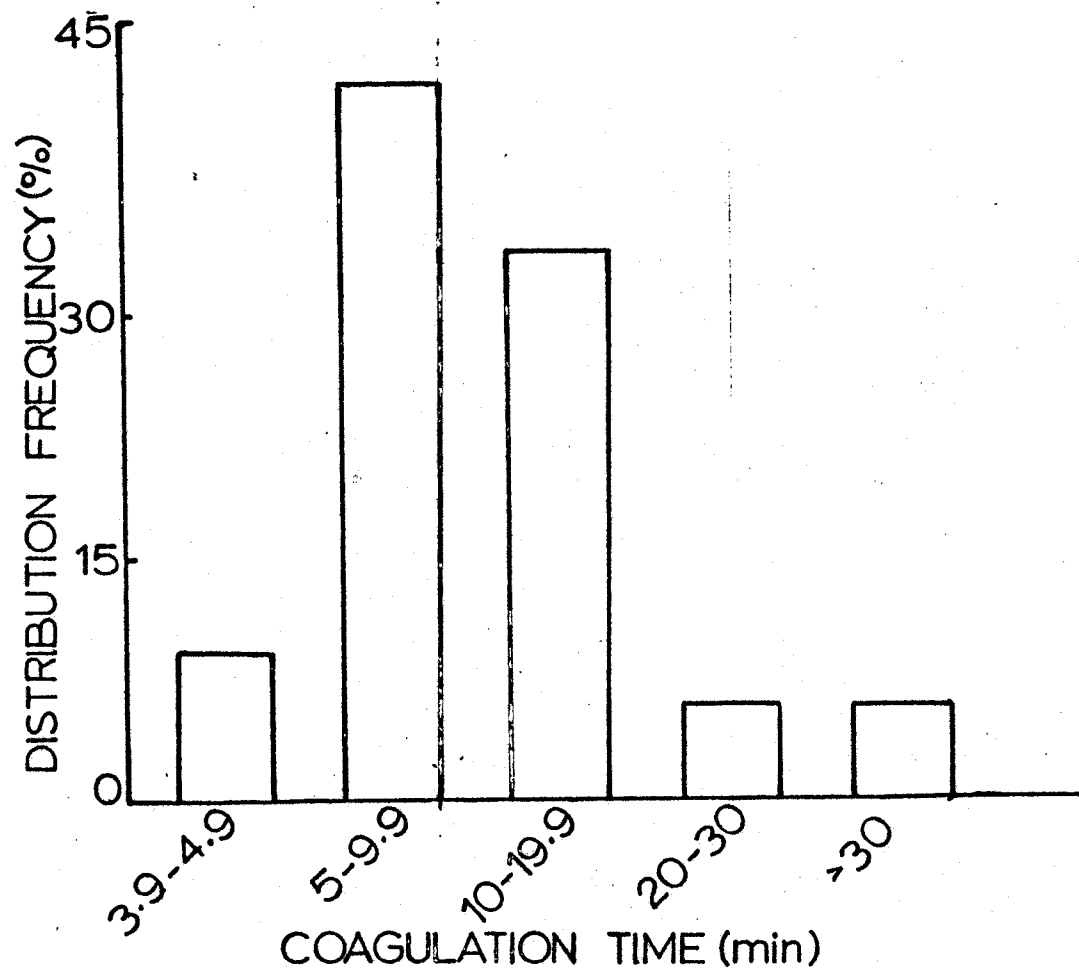


Figure 5. Distribution frequency of milk samples from individual Holstein cows according to their coagulation times.

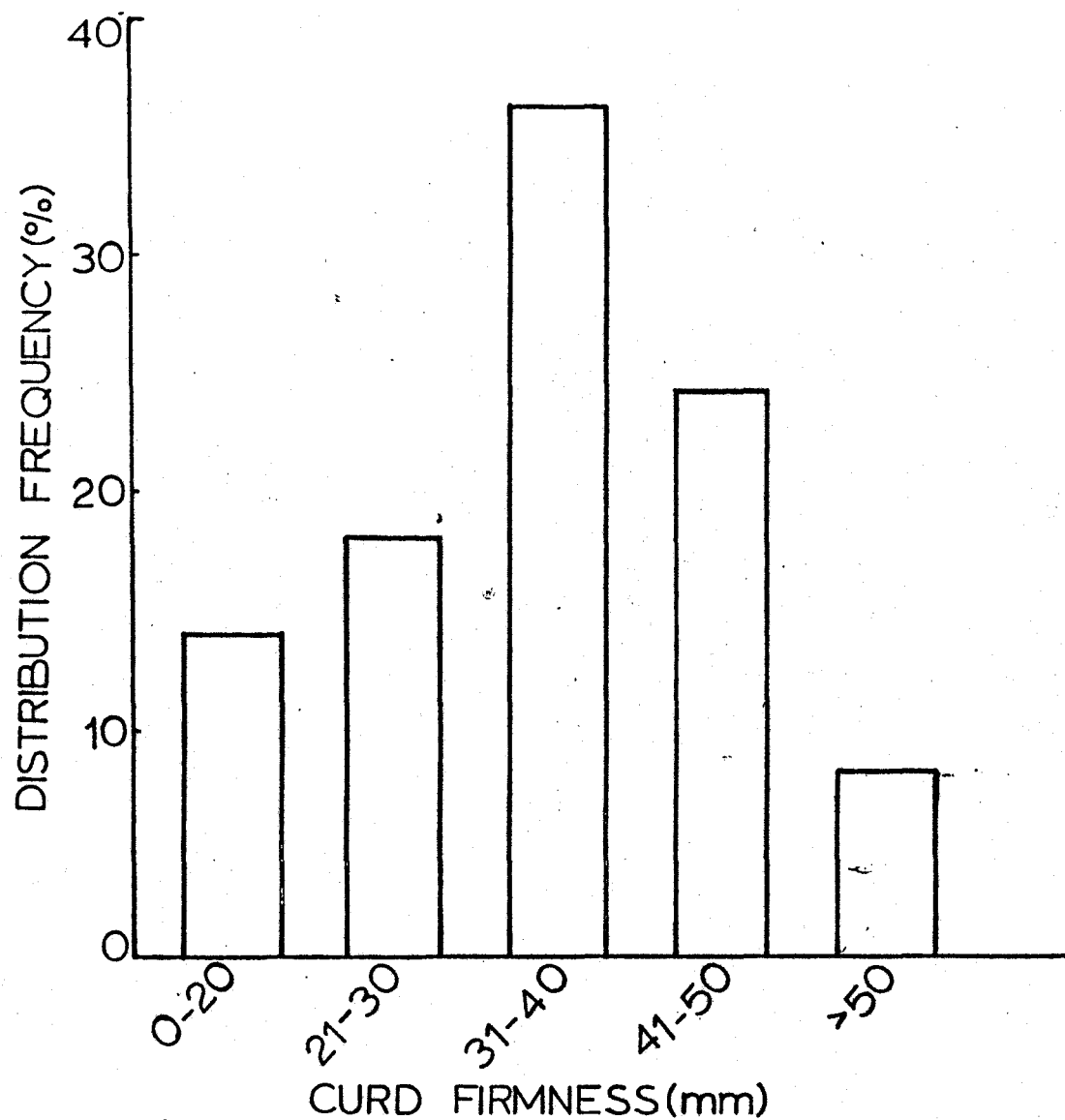


Figure 6. Distribution frequency of milk samples from individual Holstein cows according to their curd firmness. Curd firmness was determined 30 min after chymosin addition.

Table 1. Variation in curd firmness of individual cow milk samples with good-, and poor chymosin-coagulation characteristics (GCM and PCM) and also the results of blending them in 50% ratios.

GCM		PCM		50% blend	
Curd firmness (mm)	pH	Curd firmness (mm)	pH	Curd firmness after blending (mm)	pH
52	6.59	0	7.06	9	6.81
51	6.56	0	7.11	7	6.81
53	6.53	0	6.89	2	6.79
52	6.5	1	6.83	22	6.71
45	6.6	3	6.98	39	6.74
49	6.48	25	6.79	39	6.66
45	6.59	19	6.71	38	6.72
43	6.64	22	6.84	23	6.80
42	6.58	17	6.62	35	6.66
49	6.57	32	6.8	43	6.72
54	6.52	21	6.64	37	6.66
44	6.66	25	6.69	20	6.78
39	6.66	26	6.7	27	6.75
45	6.49	35	6.65	40	6.66
40	6.61	28	6.71	35	6.73
Mean	46.9	16.9	6.80	27.7	6.73
SD	4.88	12.66	.15	13.34	.06

firmness of the blends. Some blends, however, were more affected than others in manifesting the negative influence. The mean curd firmness of the blends was 27.7 mm and mean pH was 6.73. This implies that GCM samples did not adequately compensate PCM samples as hoped since their mean curd firmness was below the overall mean of 32.4 mm for the herd.

The results of coagulating blends of all 15 GCM, all 15 PCM and 50% of each blend together are shown in Table 2. The effect of having equal proportions of GCM and PCM samples in a milk blend was evident. The latter overwhelmed the former and resulted in a non-coagulating condition. Milk of similar composition can be approximated when a predominant proportion of cows in a herd is in late lactation. Previous work (21) demonstrated that in one herd 38% of milk samples obtained from cows one month prior the end of their lactation period did not coagulate in 30 min. Bulk milk with similar or approximate characteristics would need modification, otherwise, the high protein and fat of such late lactation milk (13) would be under-utilized and produce less yield of cheese since substantial losses would occur in whey (11). Apart from yield loss, Lyall (17) and Lawrence and Gilles (16) indicated that Cheddar cheese made towards the end of the cheese-making season, when all cows are in late lactation, has unsatisfactory properties. High moisture is usually associated with such cheese (17) and the initial cheese pH tends to be high (16). Overall quality also deteriorates faster than cheese made with normal milk and

Table 2. Variation in coagulation properties of pooled milk from individual cows. Curd firmness was determined 30 min after chymosin addition.

Substrate	Coagulation time (min)	Curd firming time(min)	Curd firmness (mm)	pH
Pool of all the best coagulating samples	7.3	4.4	45	6.65
Pool of all the worst coagulating samples	>30	>30	0	6.9
50% each of both pools	>30	>30	0	6.79
Berridge	4.3	2.9	55	6.3
12% non fat dry milk	15.9	8.1	33	6.59

results in a shorter shelf life (11). Shorter shelf life of cheese made with such milk might be attributed to greater acid development as a result of increased lactose content in the higher moisture cheese. Further enzymic degradation of casein by indigeneous milk enzymes in finished cheese is also possible. Pasteurization of milk activates plasmin activity (2).

#### Effect of modification of individual samples

The effects of reducing milk pH, adding .02%  $\text{CaCl}_2$ , and reducing chymosin concentration, are summarized in Table 3. The pH difference between unmodified GCM and unmodified PCM was quite wide (Table 1) considering the effect such a

difference has on enzyme activity (Figures 3 and 4).

Reducing the pH of each sample to 6.3 before coagulation caused remarkable decrease in coagulation time but not in curd firmness for both groups. After reducing pH of the PCM samples, two curd firming patterns were evident (Figure 7 (a)). Either (i) the curds began to firm but collapsed before the end of 30 min (samples B and D) or (ii) samples coagulated at higher chymosin concentration but did not firm in 30 min after chymosin addition (samples A and C). Four possibilities account for non gelation and gel decay observed at this pH:

1. Extreme proteolysis as is common with some protease enzymes other than chymosin (19), but chymosin was used in this experiment.
2. Extreme plasmin activity resulting in the conversion of some  $\beta$ - and  $\alpha_s$ -casein into soluble proteose-peptones,  $\gamma$ - and  $\lambda$ -caseins as observed in late lactation milk (2,3,14).
3. Inappropriate salt balance (12) which might have resulted in poor casein micelle aggregation.
4. Autoclaved milk exhibited an identical coagulation pattern (Wright and Richardson, 1983, unpublished) as those exhibited by samples that coagulated but never firmed. This suggests that the caseins were not very accessible to crosslinking by calcium phosphate, or they contained high amounts of unidentified, minor proteins as was observed in late lactation milk (3).

Table 3. Effects of adjustments of some cheesemaking parameters on mean chymosin coagulation properties of milk samples from individual cows. The pH of the samples was reduced to 6.3 with lactic acid before coagulation in the Formagraph.

Parameter(s) adjusted	Good coagulating samples			Poor coagulating samples		
	Coagula- tion time (min)	Curd firm- ing time (min)	Curd firm- ness (mm)	Coagula- tion time (min)	Curd firm- ing time (min)	Curd firm- ness (mm)
Unmodified samples	6.5 ±2.3	4 ±1.3	48.9 ±4.9	15.3 ±6.5	9.5 ±2.6	16.9 ±12.7
pH reduct- ion	2.9 ±.7	2.1 ±.5	48.2 ±5.0	5.9 ±5.3	3.2 ±1.1	19.5 ±17.5
pH reduct- ion + .02% CaCl <sub>2</sub> addition	3.8 ±1.7	2.3 ±.7	50.3 ±7.5	5.6 ±7.5	3.4 ±1.5	21 ±14.8
pH reduct- ion + .02% CaCl <sub>2</sub> addition + reduced chy- mosin conc.	3.3 ±2.2	2 ±1	50.1 ±6	4.8 ±2.2	3.7 1.6	31.6 ±15.6



Effect of salt balance was considered by adding .02%  $\text{CaCl}_2$  at a reduced pH of 6.3 before coagulation (Table 3). This did not adequately increase curd firmness. Thus, salt balance does not seem as important as pH in accounting for poor coagulation of PCM.

Insignificant increase in curd firmness of Berridge substrate observed by increasing chymosin concentration at decreased pH in the preliminary experiment suggested that the same curd firmness values would be obtained at lower chymosin concentration as at higher chymosin concentration in milk samples at decreased pH. Shorter coagulation times would, however, be obtained at high chymosin concentration when compared with coagulation times obtained at lower chymosin concentration. Chymosin concentration was then reduced to half its initial concentration (from .4 to .2 RU/mL). The result is also shown in Table 3 and Figure 7. A tremendous increase (86.9%) in curd firmness was observed among the PCM samples when all the 3 cheese making modifications (pH reduction, addition of .02%  $\text{CaCl}_2$ , and reducing chymosin concentration) were used at the same time. The PCM samples when unmodified were 65% weaker in curd firmness than the unmodified GCM. After all 3 modifications of both groups, only 37% difference was apparent. Improvement in curd firmness for GCM after the 3 modifications was marginal 3%. The improvement observed for PCM as a result of reducing chymosin concentration was minimal curd disintegration. This agrees with the

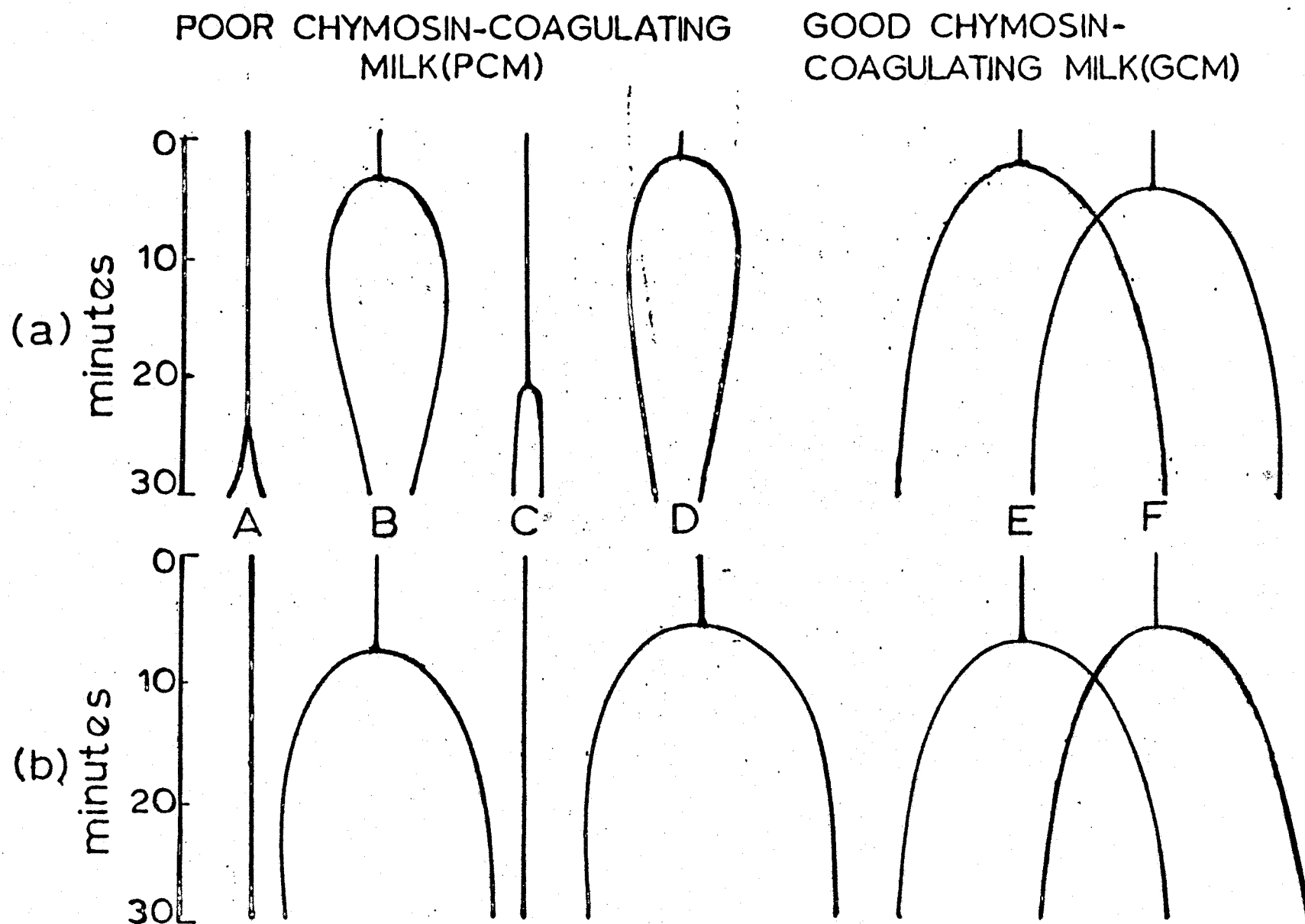


Figure 7. Effect of varying chymosin concentration on the coagulation properties of good-, and poor chymosin-coagulating individual cow milk samples adjusted to pH 6.3 with lactic acid and .02%  $\text{CaCl}_2$  added; (a) coagulated with 0.4 RU/mL of chymosin (b) coagulated with 0.2 RU/mL chymosin.

preliminary observation that high chymosin concentration at reduced milk pH had the tendency to cause proteolysis of the caseins.

Carpenter and Brown (5) showed that addition of  $\text{CaCl}_2$  to milk caused more incorporation of soluble caseins into casein micelles and hence led to increased content of total casein that could be measured by size exclusion chromatography. Ali et al. (1) showed that tempering ( $60^\circ\text{C}$  for 30 min) milk that had been in cold storage for 48 h caused greater incorporation of soluble caseins into micelles. When two variables only were considered (adding .02%  $\text{CaCl}_2$ , and tempering milk at  $37^\circ\text{C}$  for 90 min) coagulation properties of PCM did not improve substantially. Berridge substrate tempered at different temperatures for different durations (Table 4) showed that tempering at  $37^\circ\text{C}$  for 90 min caused better coagulation than tempering at  $60^\circ\text{C}$  for 30 min. Storry and Ford (24) also showed that coagulum strength was markedly reduced by increased temperature. These observations partly suggest that increased plasmin degradation of  $\beta$ - and  $\alpha_s$ -caseins to soluble proteose peptone, and to  $\gamma$ - and  $\lambda$ -caseins (2) in late lactation milk (3) caused substantial losses of caseins that would have participated in curd formation.

Storry et al. (25) correlated high curd firmness with increased  $\alpha_s$  - and  $\beta$ -caseins. This suggests that low curd firmness in PCM is partly due to casein degradation into protein fractions that do not participate in curd formation

Table 4. Effect of different temperatures and tempering times on the coagulation properties of chymosin-coagulated Berridge substrate at pH 6.3.

Temperature and time	Coagulation time (min)	Curd firming time(min)	Curd firmness (mm)
37°C for 90 min	4.4	2.9	54.5
40°C for 75 min	5.9	3.9	49
45°C for 60 min	6.7	3.9	50
50°C for 45 min	7.9	4.5	47.5
60°C for 30 min	9.0	5.4	43

and poor curd formation has been associated with late lactation milk (6,21). Fox and Mulvihill (10) also indicated that rennet curd formed in late lactation milk shows poor syneresis characteristics. The curd disintegration observed at higher chymosin concentrations in PCM (Figure 7) but less apparent in GCM suggests that the caseins present in the former were more vulnerable to proteolysis by chymosin or synergism of chymosin and plasmin activities. Less susceptibility of Berridge substrate (which consists of pooled normal milk, spray-dried and reconstituted in .01 M  $\text{CaCl}_2$  solution) in the preliminary experiment to such degradation supports this assertion. Further research is needed to explain the relative distribution of caseins in the PCM in order to detect if abnormal content of  $\alpha_s$ - and  $\beta$ -caseins is partly responsible for their overall low curd

firmness. More investigation is needed to identify interactions between the coagulation properties of GCM and PCM with different pH, temperature and chymosin levels. Such interactions, if significant, will be highly applicable in optimizing the variables for obtaining optimum curd firmness and product yields.

## SUMMARY

Individual Holstein cow milk samples were selected for good and poor chymosin-coagulation characteristics. The effect of pH adjustment, addition of .02%  $\text{CaCl}_2$ , and variation in chymosin concentration on coagulation properties of good and poor-coagulating samples was evaluated. Pooling 50% good and 50% poor samples did not improve the average coagulation properties of the poor samples. Reducing milk pH to 6.3 caused a significant decrease in coagulation time but a less marked increase in curd firmness. The greatest increase in curd firmness was obtained by combining reduction of milk pH, addition of .02%  $\text{CaCl}_2$  and reduction of chymosin concentration. Higher chymosin concentration at reduced pH decreased coagulation time without substantially increasing curd firmness. Curd disintegration was more apparent at higher chymosin concentrations in the poor-coagulating samples.

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EVALUATION AND IMPROVEMENT OF COAGULATION PROPERTIES  
OF MILK FOR MANUFACTURING

II. Interactions of calcium, pH, and chymosin during milk  
coagulation.

by

Leslie M. Ukigbo

## INTRODUCTION

Much has been published on factors which affect enzymic milk coagulation. Most publications, however, emphasize main factor effects. Information on interactions of milk coagulation factors is limited (2,4,8,9,10,13). Furthermore most authors base their reports on bulk milk used as substrate. The use of bulk milk conceals some information because milk samples with good and poor chymosin-coagulation characteristics have shown different interactions with some of these factors (17). It is desired to know how different types of coagulating milk (11,16,17) respond to these factors. This would enable proper adjustment of the levels of these factors during seasons or stages of lactation when coagulation properties of milk are different in order to maximize yield and improve the quality of cheese.

## METHODS AND PROCEDURES

### Selection of milk samples

Individual milk samples with good and poor chymosin-coagulation characteristics (GCM and PCM) (17) were collected from six Holstein cows in the Utah State University dairy herd. One liter of milk collected from each cow was from a well-stirred, complete, evening-milking. Milk samples were stored overnight at 4°C to maintain a uniform temperature history (15). Three temperatures, 32, 35 and 37°C were confounded with cows (1 GCM and 1 PCM sample were confounded with each temperature level ); two  $\text{CaCl}_2$  levels 0 and .02% added; three pH levels, 6.3, 6.4 and 6.5; and five levels of chymosin, .1, .2, .3, .4 and .5 rennin units (RU) per milliliter (mL) of distilled water were selected (9,12,17,24). All levels of the unconfounded factors were then tested by varying one factor level at a time. The Formagraph (12) was used to record the coagulation properties of the milk.

### Statistical analyses

The factorial design utilized in the analysis of variance (14,21) was a 2 (coagulating milk type) x 2 (presence or absence of added calcium) x 3 (pH levels) x 5 (rennin concentration) x 3 (temperature). Tukey multiple comparison (14) was used to compare mean coagulation properties produced by each factor level.

## RESULTS AND DISCUSSION

### Effect of factor levels

All main factor effects were significant ( $p < .0001$ ) in altering both coagulation time (CT) and curd firmness (CF) (Tables 5 and 6) which agrees with previous reports (2,4,6,8,9,10,12,18,24,25).

### Multiple comparisons of factor levels

Tukey multiple comparisons (14) of mean effects of factor levels showed that lower levels of chymosin (.1,.2, and .3 RU/mL) were significantly different from each other in altering CT (Table 7). Higher chymosin concentrations (.4 and .5 RU/mL), however, produced shorter CT which were insignificantly different from each other, but significantly different from the lower levels. The comparisons of mean CF produced by different levels of chymosin was different. The mean CF produced by .1 RU/mL was significantly lower than CF produced by .2 to .5 RU/mL levels. In practise, chymosin concentrations greater than .2 RU/mL might not be necessary for adequate curd firmness to be produced 30 min after chymosin addition. Olson and Bottazzi (18) indicated that at high chymosin concentrations, in the presence of added phosphoric acid, curd firming rate was highly retarded. Shorter CT observed at higher chymosin concentrations could erroneously suggest that higher CF would be produced at high levels of chymosin. Care must be taken, however, to avoid

Table 5. Factorial analysis of variance of chymosin  
coagulation time of milk from individual cows. The  
 $R^2 = .993$  and  $CV = 10.1\%$ .

Source	DF	MS	F	Probability
Rennin unit (RU)	4	601.4	1875.2	.0001
Coagulating milk type (CMT)	1	145.6	454.1	.0001
RU*CMT	4	6.5	20.1	.0001
pH	2	154.5	481.8	.0001
RU*pH	8	12.5	39.1	.0001
CMT*pH	2	3.7	11.4	.0001
Calcium chloride(Ca)	1	111.2	346.9	.0001
RU*Ca	4	9.7	30.3	.0001
CMT*Ca	1	5	15.7	.0002
pH*Ca	2	7.4	22.9	.0001
Temperature (Temp)	2	27.8	86.5	.0001
RU*Temp	8	2.6	8.2	.0001
CMT*Temp	2	.2	.6	.5600
pH*Temp	4	2.9	8.9	.0001
Ca*Temp	2	2.1	6.3	.0029
RU*CMT*Temp	8	.5	1.4	.2143
RU*pH*Ca	8	4.8	14.9	.0001
RU*CMT*pH	8	1.4	4.2	.0003
RU*pH*Temp	16	.4	1.3	.2183
RU*CMT*Ca	4	.1	.2	.9550
CMT*pH*Temp	4	1.6	5.1	.0011
CMT*pH*Ca	2	5.2	16.2	.0001
CMT*Ca*Temp	2	1.5	4.5	.0145
pH*Ca*Temp	4	1.1	3.2	.0180
Error	76	.3		

Table 6. Factorial analysis of variance of curd firmness of milk from individual cows 30 min after chymosin addition. The  $R^2 = .953$  and  $CV = 9.7\%$ .

Source	DF	MS	F	Probability
Rennin unit (RU)	4	330.4	17.3	.0001
Coagulating milk type (CMT)	1	18548.1	969.7	.0001
RU*CMT	4	24	1.3	.2962
pH	2	256.9	13.4	.0001
RU*pH	8	90.4	4.7	.0001
CMT*pH	2	1.9	.1	.9084
Calcium chloride(Ca)	1	154.6	8.1	.0057
RU*Ca	4	177.9	9.3	.0001
CMT*Ca	1	2.5	.1	.7189
pH*Ca	2	29	1.5	.2266
Temperature (Temp)	2	771.2	40.3	.0001
RU*Temp	8	19.1	1.0	.4438
CMT*Temp	2	1654.9	86.5	.0001
PH*Temp	4	25.7	1.3	.2621
Ca*Temp	2	81.4	4.3	.0177
RU*CMT*Temp	8	26.6	1.4	.2141
RU*pH*Ca	8	65.2	3.4	.0021
RU*CMT*pH	8	25.3	1.3	.2448
RU*pH*Temp	16	16.5	.9	.6123
RU*CMT*Ca	4	32	1.7	.1653
CMT*pH*Temp	4	93.4	4.9	.0015
CMT*pH*Ca	2	12.1	.6	.5354
CMT*Ca*Temp	2	84.9	4.5	.0150
pH*Ca*Temp	4	8.1	.4	.7938
Error	76	19.1		

Table 7. Tuckey multiple comparison of mean chymosin coagulation properties and rennin units. Means with the same letter are not significantly different at alpha level = .05.

COAGULATION TIME (min):		
Grouping	Mean	Rennin units/mL
A	12.2	0.1
B	6.4	0.2
C	4.2	0.3
D	2.9	0.4
D	2.4	0.5
CURD FIRMNESS (mm) <sup>1</sup> :		
A	47.3	0.5
A		
A	46.8	0.2
	B	
	B	0.3
	B	
	B	0.4
C	39.9	0.1

<sup>1</sup>Curd firmness values 30 min after chymosin addition.

adding very low chymosin concentrations to high pH milk. Thus, if milk pH is adequately reduced before chymosin addition, optimum CF can be produced in 30 min after chymosin addition.

Comparisons of mean CT produced by different pH levels (Table 8) showed that each pH level was significantly different in affecting CT, in agreement with previous reports (8,17,20,24). Thus shorter CT was produced at lower pH. The effects of pH levels on CF were different. Insignificantly different CF values were observed at pH 6.3 and 6.4 but were different from the CF value at pH 6.5. This indicates that reduction of milk pH to at least 6.4 would assure production of firmer curds (3,8,15). This measure would be most valuable in PCM (11,15,17), for example when dairy cows are predominantly in late lactation (Appendix 2). Lower pH values accelerate enzyme cleavage of caseins (5,20). The PCM samples have high pH (17) and their casein would be slowly cleaved by chymosin unless the pH is reduced. A previous report (17) indicated that optimum CF could be produced at pH 6.3. Even though the mean CF value was higher at this pH, it was not different from the mean CF value at pH 6.4 (Table 7). Lactic starter activity is required to achieve significant pH reduction before chymosin is added to cheese milk. Williamson and Speck (26) and Emmons et al. (3) also emphasized the importance of pH reduction for optimum curd development.



Table 8. Tukey multiple comparison of mean chymosin coagulation properties and pH. Means with the same letter are not significantly different at alpha level = .05.

COAGULATION TIME (min):		
Grouping	Mean	pH
A	7.2	6.5
B	5.6	6.4
C	4.1	6.3
CURD FIRMNESS (mm) <sup>1</sup> :		
A	46.6	6.3
A		
A	46.1	6.4
B	42.9	6.5

<sup>1</sup>Curd firmness values 30 min after chymosin.

The effects of different temperature levels on coagulation properties is shown in Table 9. Mean coagulation times produced by the three temperature levels were not significantly different from each other. A different relationship was observed between CF and temperature where 37°C produced significantly higher CF than both 32 and 35°C. These observations support making cheese at higher temperatures (23). Higher temperatures would increase collisions of chymosin-treated hydrophobic casein micelles and hence a more rapid aggregation of micelles resulting in a faster gelling rate (2,9). Much higher temperatures will be unfavorable because of denaturation of  $\kappa$ -lactoglobulin which will bind  $\kappa$ -casein resulting in a soft curd (20).

Table 9. Tukey multiple comparison of mean chymosin coagulation properties and temperature. Means with the same letter are not significantly different at alpha level = .05.

COAGULATION TIME (min):		
Grouping	Mean	Temperature (°C)
A	6.3	32
A		
A	5.7	37
A		
A	4.9	35
CURD FIRMNESS (mm) <sup>1</sup> :		
A	49.4	37
B	43.5	32
B		
B	42.8	35

<sup>1</sup> Curd firmness values 30 min after chymosin addition.

Very high temperatures will also be inhibitive to the growth of mesophillic lactic starters. Use of higher temperatures for cheese making together with high temperature lactic starters will not only reduce cheese making time but would also increase yield through retention of more milk fat as a result of production of firmer curds (1,7 and Appendix 3).

#### Effects of interactions

The 2-way interactions (Table 6) which significantly affected CT included, RU\*CMT, RU\*pH, RU\*Ca, CMT\*Ca, pH\*Ca, RU\*Temp, pH\*Temp and CA\*Temp. A curvilinear relationship existed between CT and chymosin levels (Figure 8). At the lowest chymosin level (0.1 RU/mL) the difference in mean CT

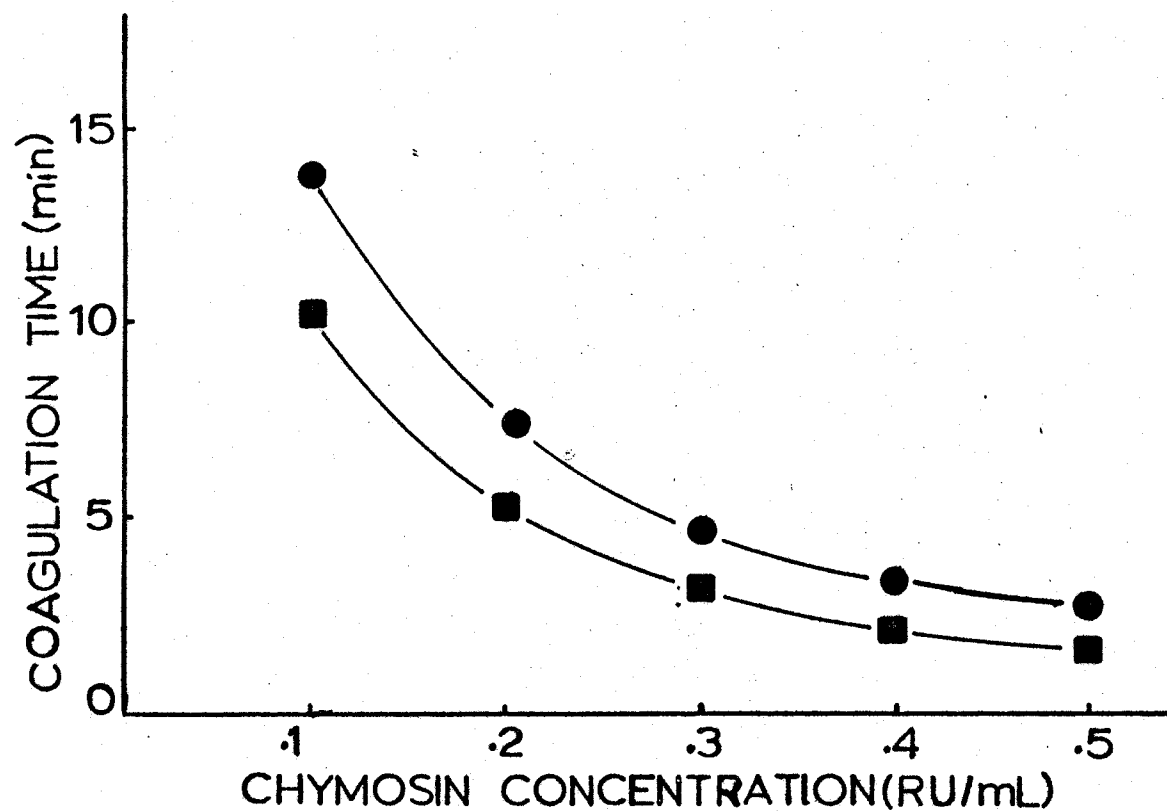


Figure 8. Interactive effect of chymosin and type of coagulating milk (■ good-, and ● poor chymosin-coagulating milk) on coagulation time.

between the two coagulating milk types (CMT) was 3.4 min, but at 0.5 RU/mL the difference in CT was 1.5 min. Thus, increasing chymosin concentrations decreased CT of the PCM more than it did for GCM. CF, however, was not significantly affected by the RU\*CMT interaction (Table 6). This indicates that when coagulation properties of milk are poor, there will be no advantage to adding more chymosin. Addition of more chymosin only decreases CT but does not significantly increase CF. CF is more important than CT in cheese making because it affects cheese yield significantly (1,7 and Appendix 3).

The interaction between chymosin and pH was significant ( $p < .0001$ ) in affecting CT (Table 5). The relationship is illustrated in Figure 9. Largest differences in mean CT between pH levels were apparent at the lowest chymosin level. At higher chymosin levels, however, pH 6.5 milk had the greatest decrease in CT when compared to pH 6.4 and 6.3 milks. Thus, higher chymosin levels produced more decrease in CT in the higher pH milk than in lower pH milks. CF in addition was significantly affected ( $p < .0001$ ) by the RU\*pH interaction (Figure 10). At 0.1 RU/mL the greatest differences were observed in mean CF of milk at different pH. But from 0.2 to 0.5 RU/mL chymosin concentrations, the differences in CF were not apparent. This agrees with the multiple range test of the effects of different chymosin levels on CF (Table 7). However, it appears that the curves of pH 6.3 and 6.4 milks had zero slopes between 0.4 and 0.5

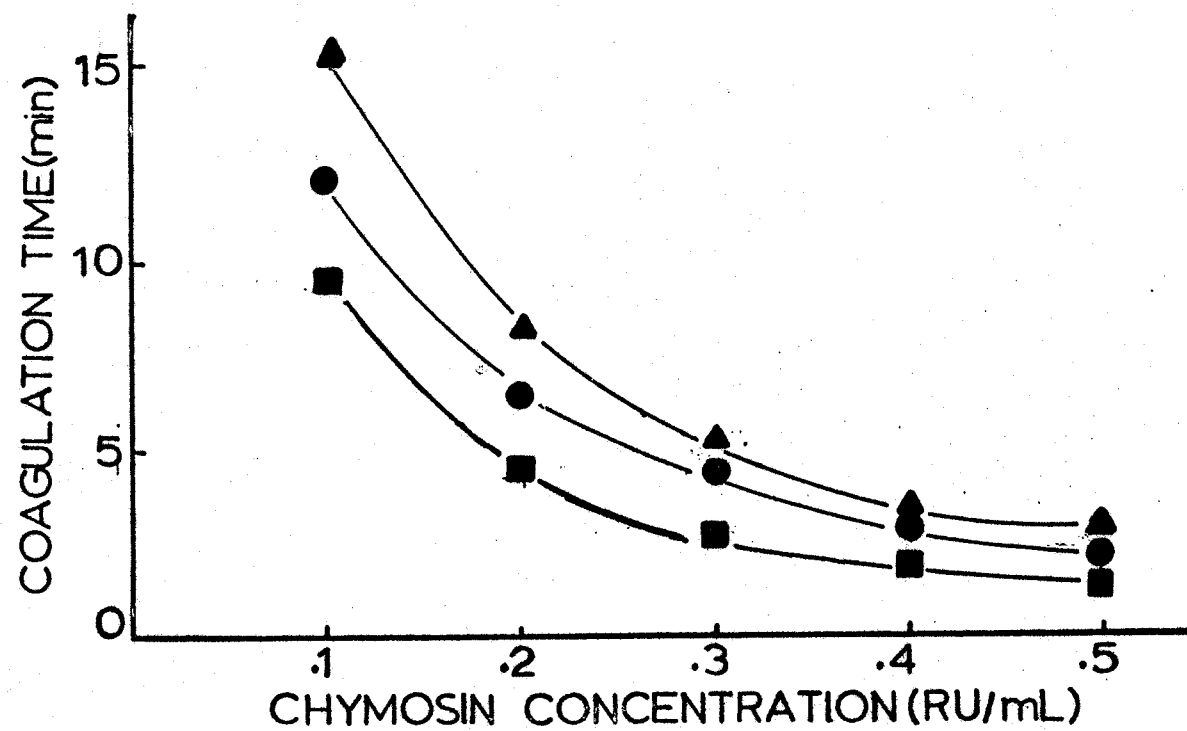


Figure 9. Interactive effect of chymosin and pH on coagulation time (■ pH 6.3, ● pH 6.4, and ▲ pH 6.5).

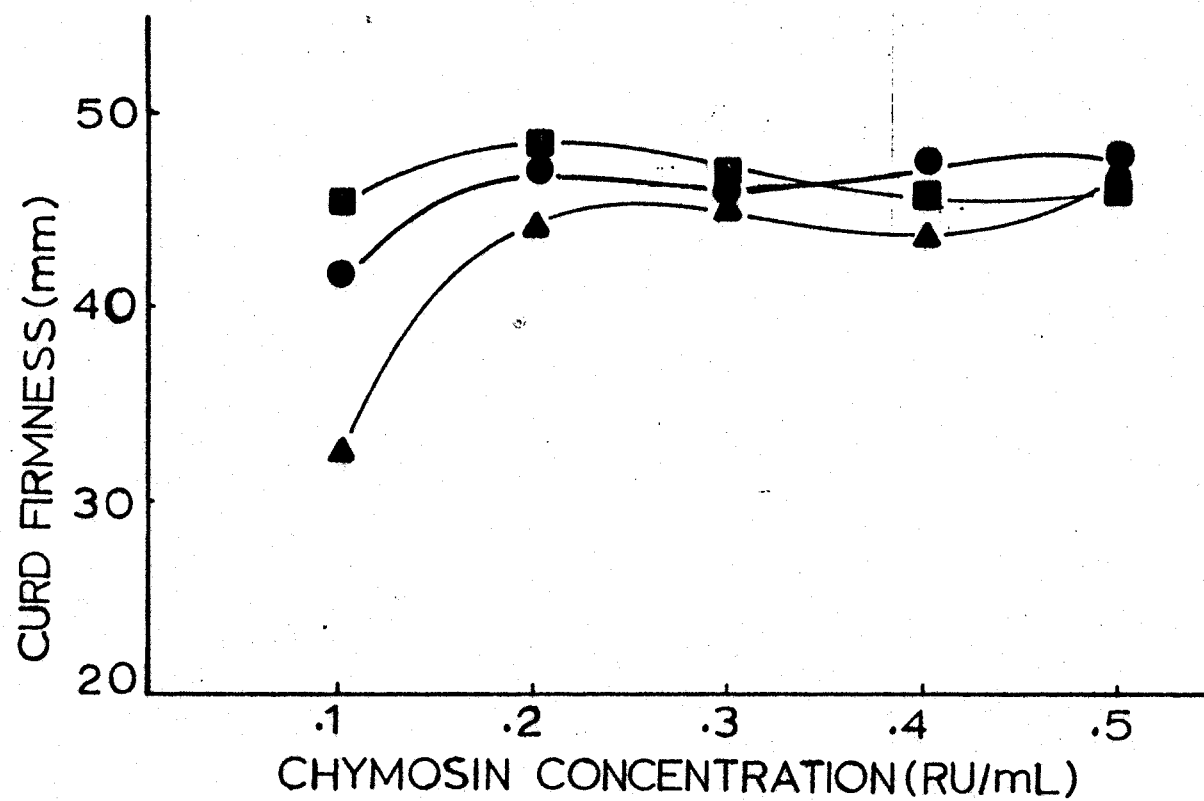


Figure 10. Interactive effect of chymosin and pH on curd firmness 30 min after chymosin addition (■ pH 6.3, ● pH 6.4, and ▲ pH 6.5).

RU/mL suggesting that additional chymosin would not have produced increased CF. On the other hand, pH 6.5 milk appeared to have the potential for increased CF at higher chymosin levels. In a previous study (17) high chymosin concentration (0.4 RU/mL) caused curd deformation or decay at pH 6.3 in PCM, but curd from the same milk was stronger at lower chymosin concentration (0.2 RU/mL). The production of firmer curds would be assured in GCM or PCM with low chymosin concentration, and low pH. Chymosin concentration of 0.2 RU/mL and pH 6.4 appears optimal for coagulating cheese milk.

The interaction between CMT and pH was significant ( $p < .0001$ ) in affecting CT (Table 5). The data plot is similar to Figure 8. The interaction between CMT and pH was, however, insignificant in affecting CF. Thus, reducing milk pH before adding chymosin increased CF for both PCM and GCM at the same rate over the stated pH range. This confirms that pH reduction is necessary before chymosin is added to cheese milk even with GCM.

The interaction between chymosin concentration and .02%  $\text{CaCl}_2$  was significant ( $p < .0001$ ) in affecting CT (Table 6). This interaction is illustrated in Figure 11. Increasing chymosin concentration reduced the need for added .02%  $\text{CaCl}_2$  (4). CF, however, was differently affected (Figure 12). At the lowest chymosin concentration (0.1 RU/mL), a wide difference was observed between the CF of milk in which  $\text{CaCl}_2$  was added. From 0.2 to 0.5 RU/mL, there

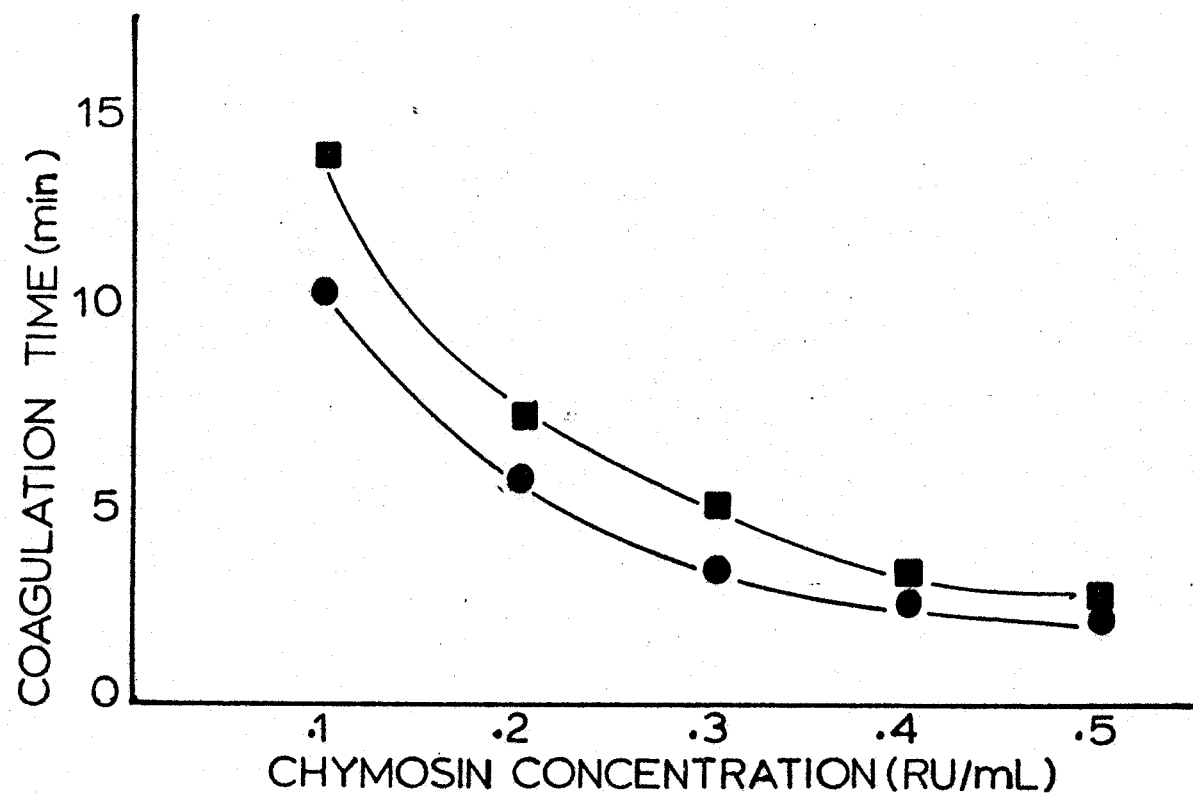


Figure 11. Interactive effect of chymosin and .02% CaCl<sub>2</sub> on coagulation time (● .02% CaCl<sub>2</sub> added, ■ no added CaCl<sub>2</sub>).



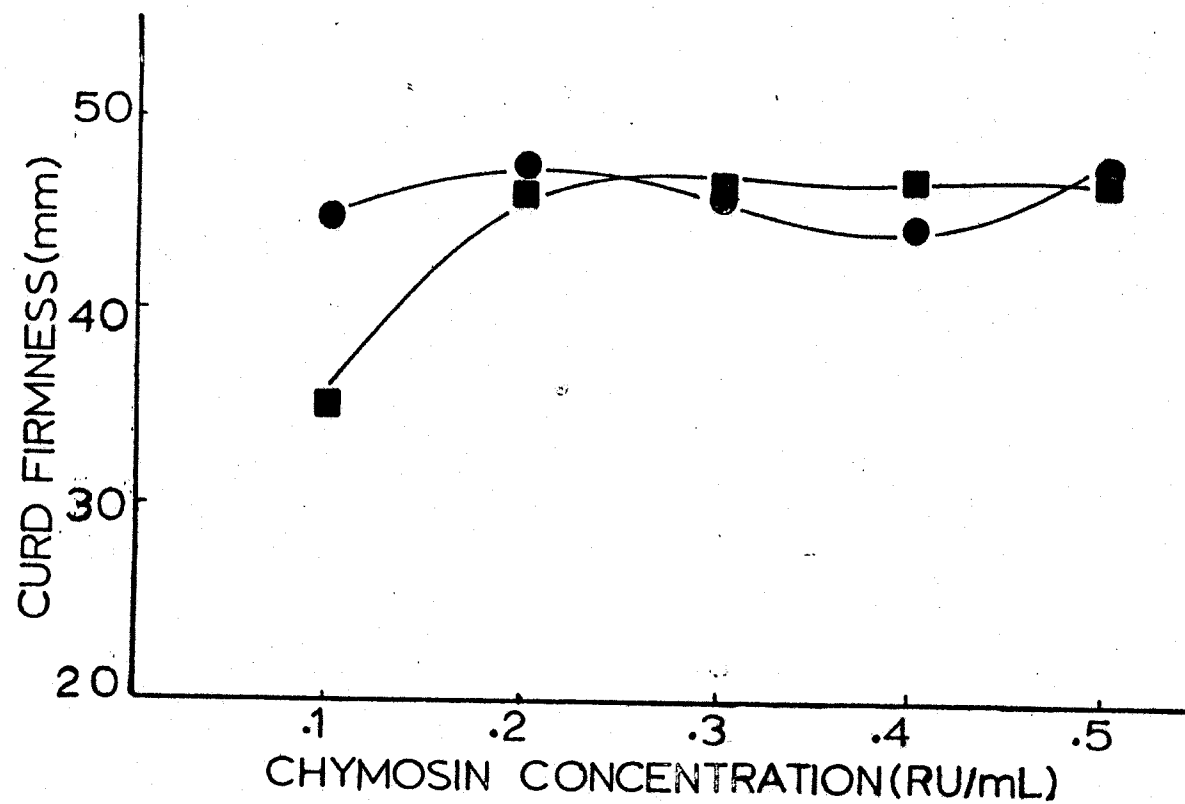


Figure 12. Interactive effect of chymosin and .02% CaCl<sub>2</sub> on curd firmness 30 min after chymosin addition (● .02% CaCl<sub>2</sub> added, ■ no CaCl<sub>2</sub> added).

was no significant difference in CF of both milks (Figure 12). This represented mean CF of milk between pH 6.3 and 6.5. Milk at pH 6.55 to 6.75 would not show similar curd firming patterns (17). For adequate CF to be produced in pH-unmodified milks, much higher level of chymosin would be needed, in addition to .02%  $\text{CaCl}_2$  addition (17). Therefore increased CF would be obtained at significantly higher cost. Lower costs would be involved if milk pH and chymosin concentration can be reduced, and no  $\text{CaCl}_2$  is added. Savello and Ernstrom (22) indicated that  $\text{CaCl}_2$  was detrimental to meltability of process cheese made from ultrafiltered milk. Added  $\text{CaCl}_2$  should be avoided if process cheese is the desired final product.

#### Optimization of curd firmness

Generally, there appeared no advantage to adding more than 0.2 RU/mL of chymosin (Table 7) for formation of adequate CF. CT was, however, significantly affected by increasing chymosin concentration. Apart from using a very low level of chymosin (0.1 RU/mL), CF was the same at all chymosin levels at 30 min when milk pH was reduced. Higher chymosin concentrations at reduced pH tended to cause curd decay and should be avoided (Figure 10). Thus, if pH is to be reduced, then low level of chymosin (0.2 RU/mL) should be added.

Addition of  $\text{CaCl}_2$  to cheesemilk to optimize the production of adequate CF 30 min after chymosin addition

appears unnecessary if other factors (pH, temperature, and chymosin concentration) can be adequately adjusted (Figure 12). Jen and Ashworth (8) indicated that addition of  $\text{CaCl}_2$  to milk partly increases CF through pH reduction, primarily due to binding of added calcium to casein micelles and release of protons. They established that if a mixture of  $\text{CaCl}_2$  and  $\text{Ca(OH)}_2$  are added to milk to keep pH constant then CF decreases compared to the situation when only  $\text{CaCl}_2$  is added.

The difficulties encountered in cheese making when coagulation properties of milk are poor can be partly arrested by adjustment of all the main factor levels that affect milk coagulation. The most significant adjustments should include reducing pH to between 6.3 and 6.4, using between 0.2 to 0.3 RU/mL of chymosin, and increasing the temperature to  $37^\circ\text{C}$ . Addition of  $\text{CaCl}_2$  might not be necessary. The above modifications would help increase CF at cutting. Increased CF at cutting increases retention of milk fat in curd (1,7), and hence increases cheese yield. Further research is needed to determine if addition of .02%  $\text{CaCl}_2$  to cheesemilk is necessary, because loss of colloidal calcium phosphate from casein micelles occurs as pH is reduced during cooking of curd. Excess loss of colloidal calcium phosphate will retard syneresis of curd, and would cause high moisture retention and mealy body in finished cheese. Addition of  $\text{CaCl}_2$  to milk might be expected to minimize the effect of this loss.

## SUMMARY

Holstein milk samples with good and poor chymosin-coagulation characteristics were coagulated in the Formagraph using different combinations of five levels of chymosin, three pH and three temperatures in the presence and absence of .02% added  $\text{CaCl}_2$ .

All the main factor effects were highly significant ( $p < .0001$ ) in altering both coagulation time and curd firmness. Multiple comparisons of mean coagulation times showed that lower levels of chymosin (.1, .2, and .3 rennin units) were significantly different in altering coagulation time but were different from higher levels (.4 and .5 rennin units). The three pH levels produced significantly different mean coagulation times. Addition of more than .2 rennin units per milliliter to milk was not necessary for adequate curd firmness to be produced 30 minutes after chymosin addition if milk pH was reduced to at least 6.4. Addition of .02%  $\text{CaCl}_2$  to milk was not necessary for adequate curd firmness to be produced 30 min after chymosin addition if other milk coagulation factors (pH,  $\text{CaCl}_2$ , and temperature) were adequately adjusted. Higher temperature ( $37^\circ\text{C}$ ) at reduced milk pH produced firmer curds.

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EVALUATION AND IMPROVEMENT OF COAGULATION PROPERTIES  
OF MILK FOR MANUFACTURING

III. Casein composition of cow's milk of different chymosin  
coagulation properties.

by

Leslie M. Okigbo



## INTRODUCTION

The relationship between chymosin coagulation properties and variability in casein composition of milk from individual cows has not been well described. Some authors (9,14,18) have shown that curd firmness is directly related to total casein in milk. Storry et al. (19) correlated high curd firmness with increased  $\alpha_s$  - and  $\beta$ -caseins of milk from different breeds and species of ruminants. Davies and Law (3) showed that casein composition was constant in mid lactation but was highly variable in early and late lactation. Barry and Donnelly (2) showed that a high level of minor, unidentified protein, and  $\gamma$ -caseins and a reduced level of  $\beta$ -casein were associated with late lactation when compared to the low level of  $\gamma$ -casein in normal milk (4), and that these changes are expected to affect syneresis characteristics of curd.

Previous studies (15) showed variation in susceptibility to deformation of milk curds from certain cows made with chymosin. Some curds were highly deformed at high chymosin concentration, were weak, and not suitable for cheese manufacture. Milk from other cows coagulated early with chymosin but the curds never firmed. All indicate that probable variations occurred in casein composition of milk from individual cows which affected their chymosin coagulation properties. The purpose of this study was to investigate possible variations in casein composition of

milk from individual Holstein cows and the effects on milk  
chymosin-coagulation properties.

## METHODS AND PROCEDURES

### Preparation of casein samples

Nine individual Holstein milk samples from the Utah State University dairy herd were selected from 33, and 17 milk samples which had good and poor chymosin-coagulation characteristics (GCM and PCM) respectively (12,15). The samples were filtered with glasswool to remove cell debris, centrifuged at  $3000 \times G$  at  $-1^{\circ}\text{C}$  to separate milk fat, and decanted to obtain skim milk. Caseins were precipitated from each 30 mL individual skim milk sample at pH 4.6 with 5 N HCl during constant stirring at  $37^{\circ}\text{C}$ . Precipitated caseins were washed with distilled water, and resolubilized with 2 N NaOH at  $20^{\circ}\text{C}$ . The pH of the sample did not exceed 8.0, and the final volume did not exceed the initial volume of milk (5). The process of precipitation and resolubilization was done twice and the final precipitate was frozen and stored at  $-20^{\circ}\text{C}$ .

### Hydroxyapatite chromatography

Laboratory scale chromatographic grade hydroxyapatite was prepared as described by Atkinson et al. (1), equilibrated and stored in 5 mM sodium phosphate, pH 6.8 buffer at  $4^{\circ}\text{C}$ . Frozen individual casein samples were thawed and 1 g of each sample was dissolved in 20 mL of 5 mM sodium phosphate in 6 M urea, pH 6.8 buffer and left overnight at  $20^{\circ}\text{C}$ . Three elution buffers which consisted of 5, 80, and 310 mM sodium phosphate in 6 M urea all adjusted to pH 6.8

with 5 N HCl were prepared (2). A 16 cm x 1.5 cm glass column was packed with hydroxyapatite, and the elution rubber tubing attached to the flowcell of a Beckman DU-8 scanning spectrophotometer (Beckman Instruments Inc., Fullerton, CA) monitor, which was interfaced with a Tektronix 4052 computer (Tektronics Inc., Beaverton, OR). The computer was programmed to plot absorbance (280 nm) of the eluent at 1 min intervals. A 3 mL sample was applied gradually on top of the column packing with a pipette and elution was commenced with 5 mM sodium phosphate in 6 M urea buffer using a peristaltic pump (Scientific Industries Inc., Bohemia, NY) adjusted to deliver .5 mL of buffer per min. After 30 mL of 5 mM buffer was used it was replaced with 25 mL of 80 mM buffer. A linear gradient of 80 to 310 mM buffer was then set up with a gradient mixer, and elution was continued for 7 h.

#### Polyacrylamide electrophoresis

Polyacrylamide (10%) gels containing 4 M urea were prepared with tris-glycine pH 8.9 buffer as described (13) and stored at 4°C no longer than 96 h (11). Five hundred milligrams of thawed casein from each individual sample was dissolved in 5 mL of 6 M urea and was mixed with a Vortex mixer (Scientific Industries, Bohemia, NY) until completely dissolved. To 250 µL of each sample was added 10 µL of .25% bromophenol blue (tracking dye). The sample was mixed again and incubated at 45°C for 3 h. During incubation, a gel was pre-run for 3 h at 50 mA in an LKB electrophoresis apparatus

(11) (LKB-Producter, Broma, Sweden) with tris-glycine, pH 8.3 electrode buffer. After incubation, 20  $\mu$ L of glycerol was added, each sample was mixed properly and 10  $\mu$ L was pipetted into a sample well in the polyacrylamide gel. Also 50 mg each of freeze-dried  $\alpha_s$ -, and  $\beta$ -casein standards (Sigma Chemical Co., St. Louis, Mo) were similarly treated. The gel was run for 10 min at 20 mA to concentrate the samples in the anode side of the wells in the gel after which the current was increased to 5mA/sample. This current was maintained until the tracking dye was monitored at the anode end of the gel, approximately 5 h. The gel was removed, fixed in a solution of trichloroacetic-sulphosalicyclic acid and methanol for 30 min, stained in comassie brilliant blue R-250 solution for 1 h, destained for 24 h in ethanol-acetic acid solution, and preserved in an ethanol-acetic acid solution (11). The main bands of the major casein variants were also over-loaded in a second gel by doubling the sample concentration in order to increase the color intensity of the minor bands for better evaluation, and electrophoresis was repeated.

Urea was added to individual 5 mL skim milk samples to a concentration of 6 M and the samples electrophoresed as described above. Acid whey samples collected after isoelectric precipitation of caseins from the individual samples were also electrophoresed.

## RESULTS AND DISCUSSION

### Variation in casein composition

Wide variations in casein composition were observed between the PCM and GCM samples. Figure 13 illustrates Formagraph (12) tracings of chymosin coagulating samples in both groups. The tracings presented in the figure were representative of all tracings not shown. Sample D (Figure 13) represents the Formagraph tracing of a typical GCM. The chromatograph of a normal clotting sample is shown in Figure 14. Sample A in Figure 13 coagulated early enough to permit adequate curd formation in 30 min, but this did not happen, indicating improper development of the secondary, non-enzymic phase of milk clotting (6). The chromatograph elution profile for casein variants of sample A is also shown in Figure 15. Abnormally high contents of para- $\kappa$ - and  $\gamma$ -1,2 and 3- caseins (a)(2), were evident when compared to the  $\kappa$ -casein and unidentified chymosin-resistant minor protein peak (b) which had a smaller peak area. Remarkable in the casein composition of this sample, were the 2 substantial minor peaks of unidentified, minor proteins (c and e). The  $\beta$ -casein was also depleted (d). The  $\alpha_s$ -casein content (f) appeared normal as evident from the peak area and when compared to a normal milk profile (2). Coagulation studies were repeated on all the milk samples in Figure 13 after storage at  $-10^{\circ}\text{C}$  for 1 mo. Sample A (Figure 13) showed a highly significant change in curd firmness, 95% reduction.

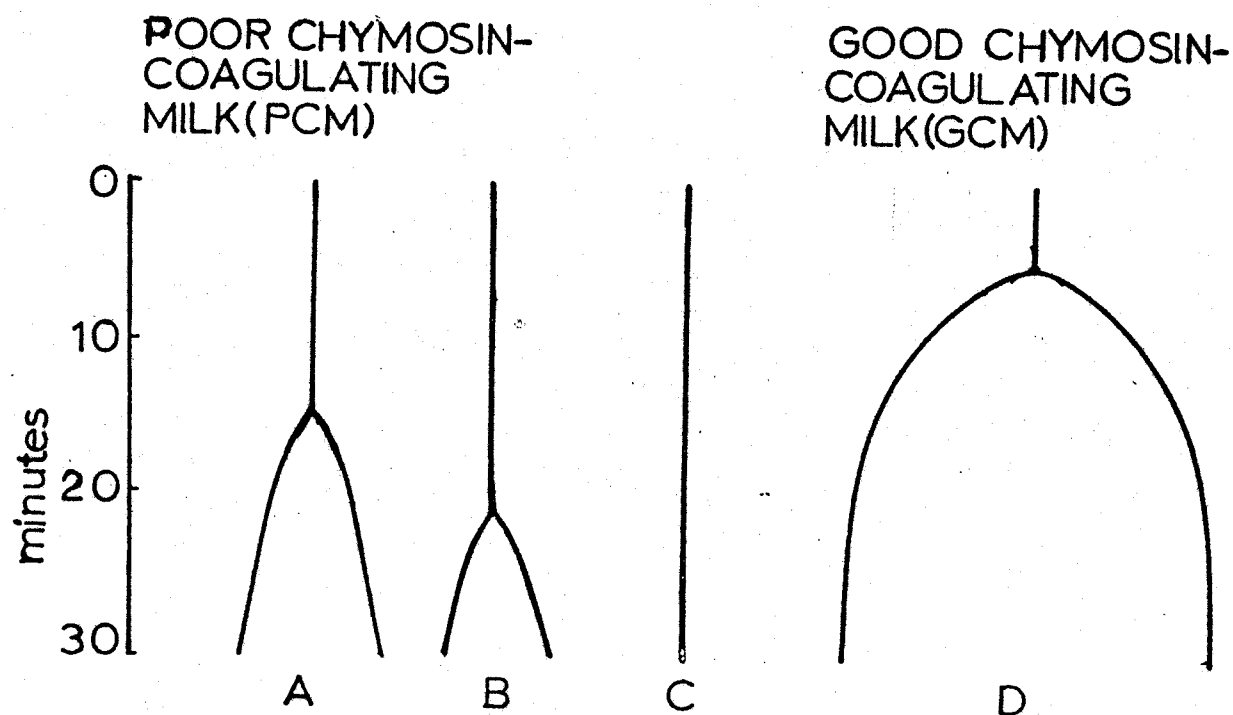


Figure 13. Formagraph tracings of good, and poor chymosin-coagulating milk samples from individual Holstein cows. Samples consisted of: A, milk which coagulated early but the curd did not firm adequately at 30 min; B, milk with long coagulation time and very weak curd; C, milk which could not coagulate 30 min after chymosin addition; D, milk with good chymosin-coagulation characteristics.

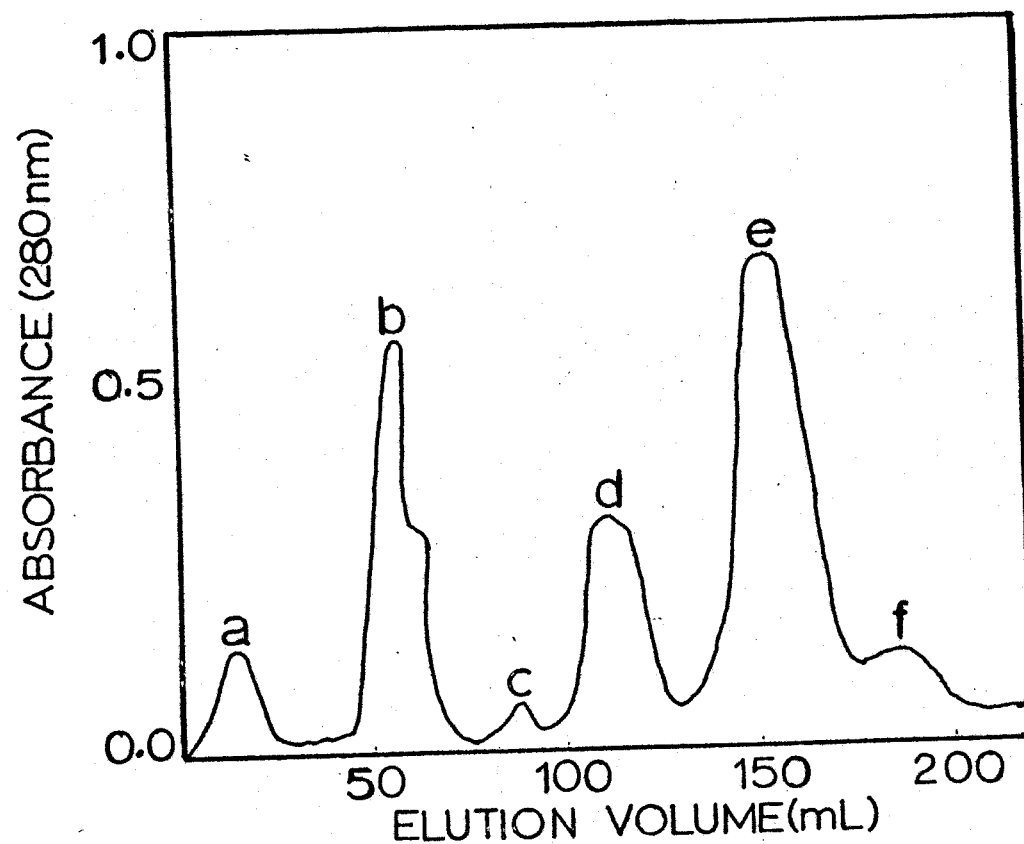


Figure 14. Hydroxyapatite chromatography of whole casein from individual Holstein cow's milk with good chymosin-coagulation characteristics. Elution peaks consist of: a,  $\gamma$  - and para- $\kappa$ -caseins; b,  $\kappa$ -casein; c, unidentified minor protein; d,  $\beta$ -casein; e,  $\alpha_{s1}$ -casein; f,  $\alpha_{s2}$ -casein.



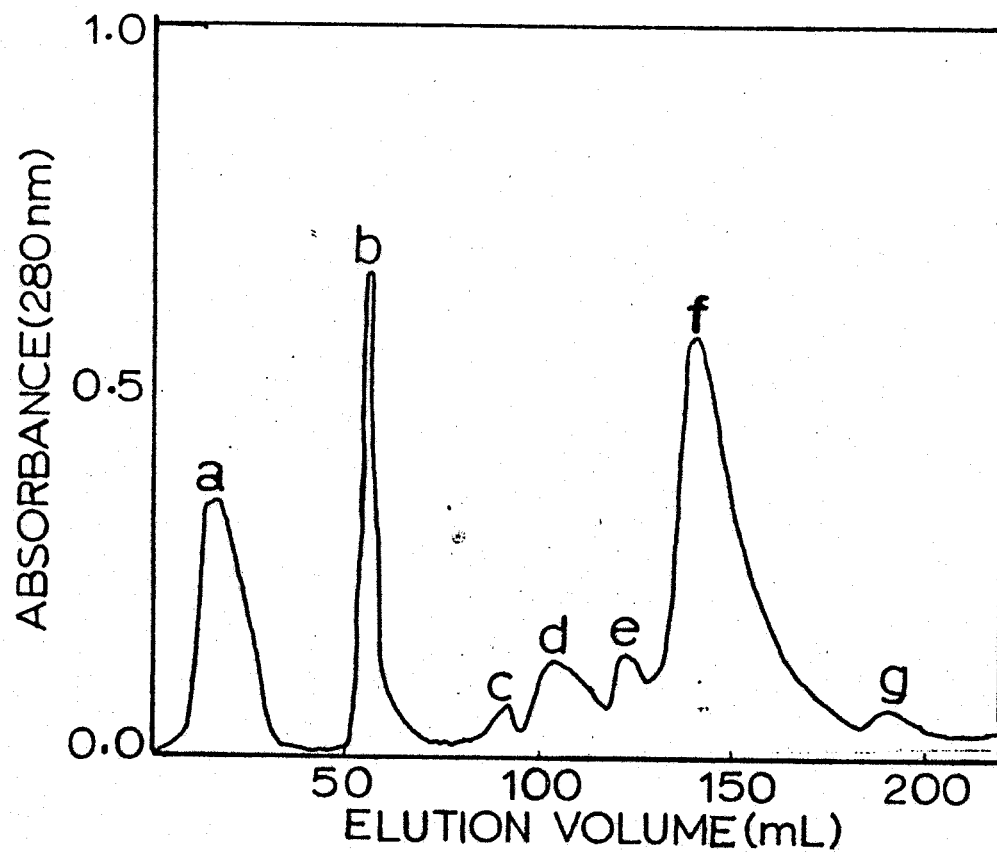


Figure 15. Hydroxyapatite chromatography of whole casein from individual Holstein cow's milk which coagulated with chymosin but its curd did not firm adequately. Elution peaks consist of: a,  $\gamma$ - and para- $\kappa$ -caseins; b,  $\kappa$ -casein; c and e, unidentified minor protein; d,  $\beta$ -casein; f,  $\alpha_{s1}$ -casein; g,  $\alpha_{s2}$ -casein.

All other samples showed less significant differences but the coagulation time of one GCM sample increased 46% and curd firmness decreased 3.6%. This suggests that accumulation of unidentified minor proteins may be responsible for inability of sample 13A to complete secondary clotting phase. A Formagraph tracing similar to sample 13A was obtained when autoclaved milk was coagulated with chymosin.  $\kappa$ -casein is known to interact with  $\beta$ -lactoglobulin in milk upon high heat treatment (16). Such milk is known to produce soft curd when coagulated with chymosin. One possibility of soft curd formation, therefore, might be the presence of these unidentified minor proteins in a significant quantity as evidenced in sample 13A.

Sample B (Figure 13) also had a casein elution profile (Figure 16) which indicated abnormal content of para- $\kappa$ - plus  $\gamma$ -1,2, and 3-caseins (a). The content of para- $\kappa$ - plus  $\gamma$ -caseins which together form minor proteins, had almost peak area equal to its  $\alpha_s$ -casein (e), the major casein. At four previous weekly samplings this sample consistently displayed the same coagulation pattern (Figure 13) and its pH was consistently near 6.8. The  $\beta$ -casein (d) content was low as was  $\kappa$ -casein (b).

Sample C (Figure 13) did not coagulate in 30 min. It had a pH of 7.1. The casein elution pattern is shown in Figure 17. All the casein variants were conspicuously low. It had an additional casein fraction (g) after the  $\alpha_s$ -casein (f), which probably was  $\lambda$ -casein. Some authors (3,4,5) have

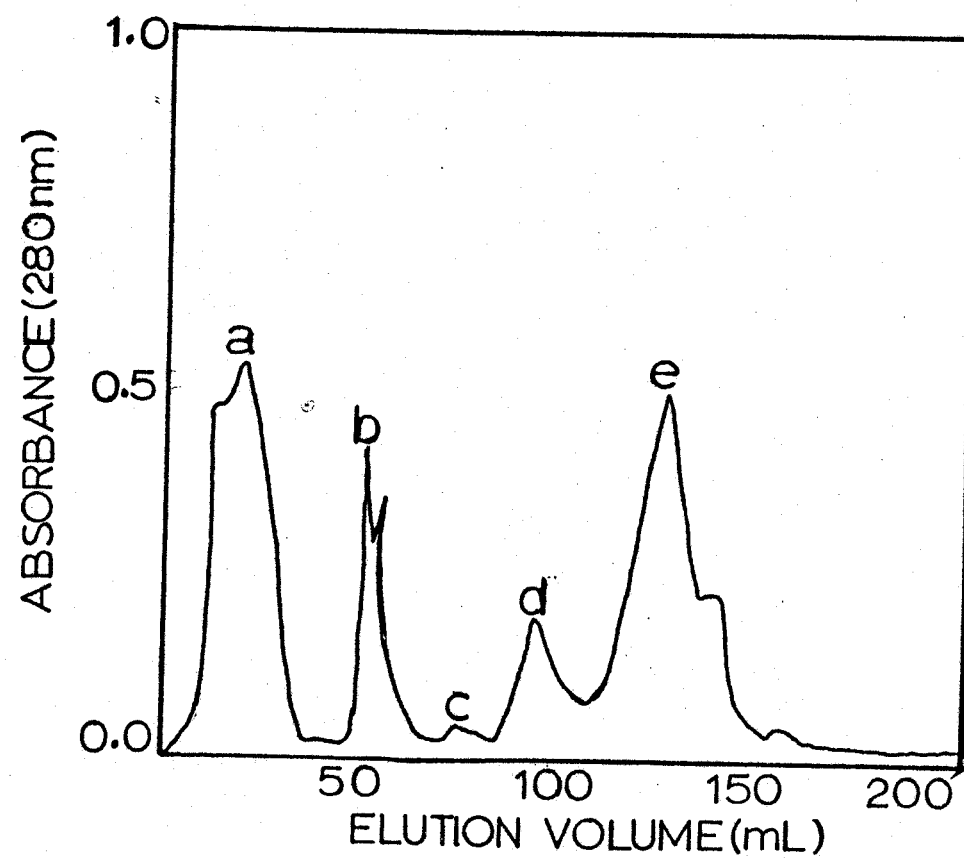


Figure 16. Hydroxyapatite chromatography of whole casein from individual Holstein cow's milk which had a long coagulation time with chymosin, and very weak curd. Elution peaks consist of: a,  $\gamma$ - and para- $\kappa$ -caseins; b,  $\kappa$ -casein; c, unidentified minor protein; d,  $\beta$ -casein; e,  $\alpha_s$ -casein.

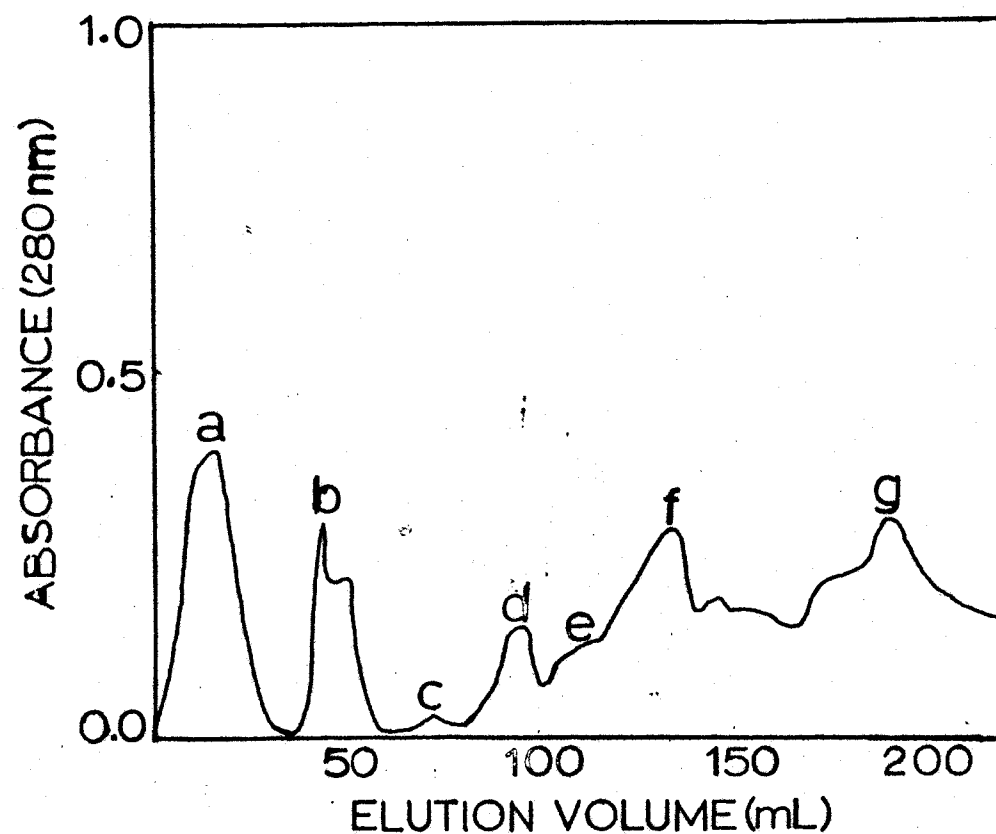


Figure 17. Hydroxyapatite chromatography of whole casein from individual Holstein cow's milk which could not coagulate 30 min after chymosin addition. Elution peaks consist of: a,  $\gamma$ - and para- $\kappa$ -caseins; b,  $\kappa$ -casein; c and e, unidentified minor protein; d,  $\beta$ -casein; f,  $\alpha_s$ -casein; g, tentatively identified  $\lambda$ -casein.

indicated that  $\alpha_s$ -casein is less susceptible to degradation by indigenous milk enzymes than  $\beta$ -casein. This observation agrees with our findings where less variability was observed in the  $\alpha_s$ -casein peaks of the samples mentioned apart from the exceptional example of the non-coagulating sample (Figure 17).

The majority of the PCM were late lactation milk samples. A remarkable characteristic they possessed was high milk pH, consistent with previous observations (14,15). Some authors (2,3,10) have shown that increased capillary permeation of blood constituents into the mammary glands, as commonly observed in late lactation, was responsible for increased content of indigenous alkaline milk proteinases of which plasmin, a proteolytic enzyme similar to trypsin (7,10), has been identified. Plasmin cleavage sites (8) do not conform to chymosin cleavage sites. Plasmin is known to be inhibited by soybean trypsin inhibitor (7). Cleavage products have been identified as para- $\kappa$ -,  $\gamma$ - and  $\lambda$ -casein-like substances in addition to proteose peptones 5 and 8 (8,20). These products have different electrophoretic mobilities than those of the major casein fractions, and precipitate together with the major caseins during isoelectric precipitation even though  $\gamma$ -caseins have isoelectric pH of about 6 (7,20).

Comparison of the contents of casein variants in the samples calculated from areas under the peaks of the chromatographs is shown in Table 10.

Table 10. Relative amounts (%) of casein variants in individual Holstein cow milk samples with good- and poor chymosin-coagulating characteristics (GCM and PCM).

Coagulating milk type	$\alpha_s$	$\beta$	$\kappa$	para- $\kappa+\gamma$	unident ified minor protein	$\lambda?$
GCM	56.3	18.3	18.8	4.8	2.2	
PCM (A)	52.6	8.7	15.6	15.8	7.0	
PCM (B)	42.2	9.7	13.2	33.9	1.0	
PCM (C)	29.7	8.3	14.0	28.4	6.6	13.5

The poor chymosin-coagulating milk samples had higher content of  $\gamma$ - and para- $\kappa$ -caseins, lower  $\beta$ - and  $\alpha_s$ -caseins than the good chymosin-coagulating milk.

Plate 1 illustrates the electrophoretic distribution of the main casein fractions. Slots 1-5 contain PCM samples. The distribution is consistent with the elution profiles obtained with hydroxyapatite chromatography. The PCM showed a lot of variability in color intensity of their  $\beta$ -casein bands and little or no variability in the  $\alpha_s$ -casein bands. The  $\beta$ -casein bands were generally lighter in color than the GCM indicating a lower content of  $\beta$ -casein. The minor casein bands of the PCM were numerous and faint and thus necessitated overloading of the main bands in another gel (Plate 2) to enable a better visualization of the minor bands. Slot 1 in Plate 2 contained the same sample as B in

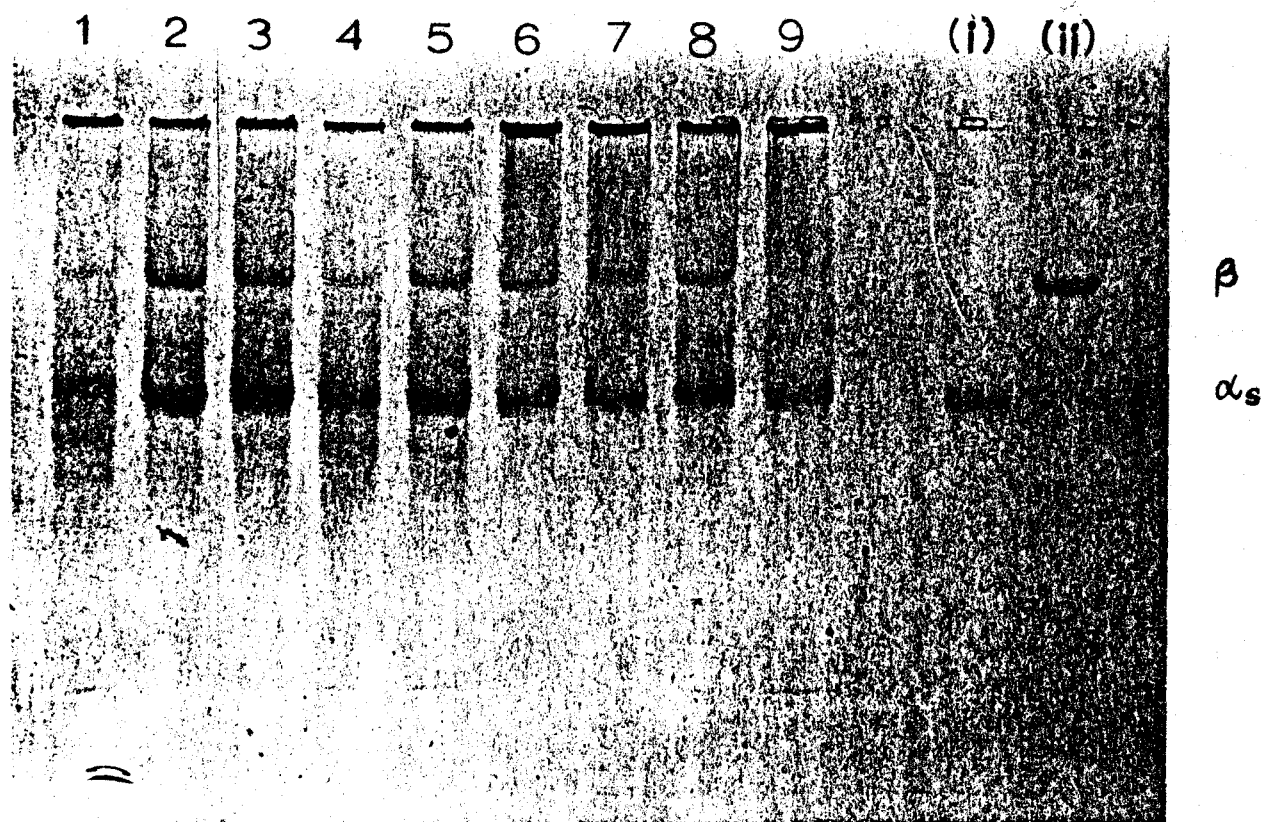


Plate 1. Polyacrylamide electrophoresis in urea gels of whole casein from individual Holstein cow's milk. Slots 1-5, and 6-9 contained poor-, and good chymosin-coagulating milk samples respectively. Slots (i) and (ii) contained  $\alpha_s$ - and  $\beta$ -caseins standards respectively.

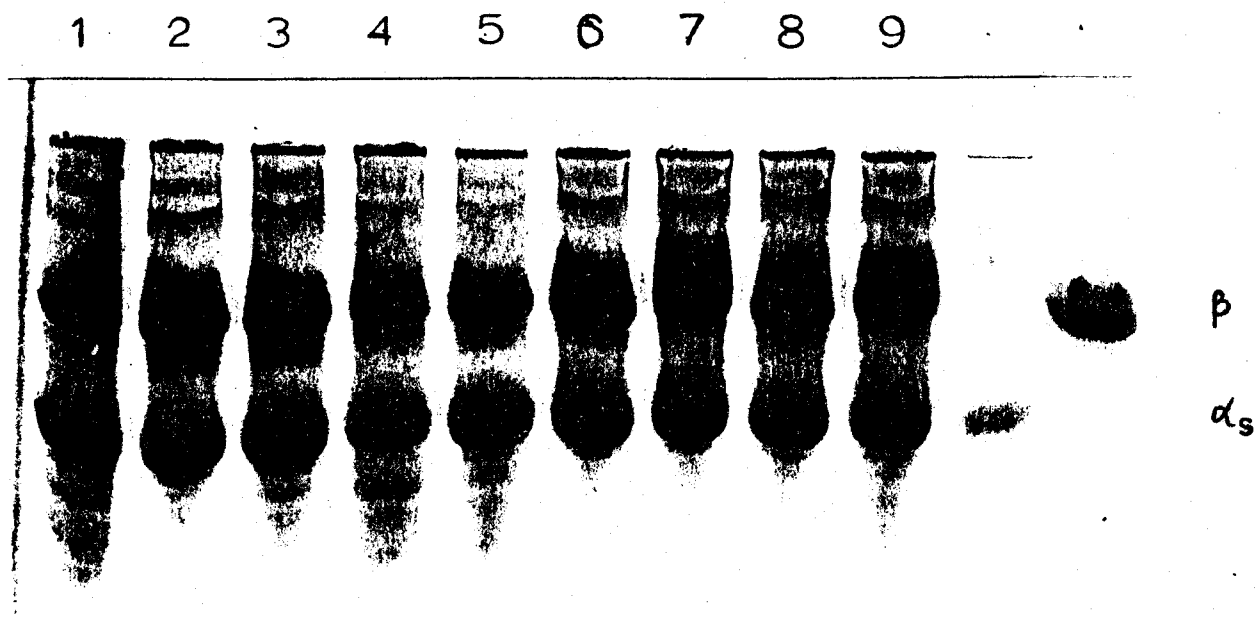


Plate 2. Polyacrylamide electrophoresis in urea gels of whole casein from individual Holstein cow's milk to show the minor casein fractions as a result of overloading the main bands. Slots 1-5, and 6-9 contained poor-, and good chymosin-coagulating milk samples respectively.



Figures 13 and 16. Innumerable minor bands in this sample agree with the large peak area of para- $\kappa$ - plus  $\gamma$ -caseins in the casein elution profile (Figure 16). Slot 4 in Plates 1 and 2 contained the sample which did not coagulate in 30 min (C in Figure 13). Absent in these plates for this particular sample was an intensely colored band in each plate that was close to the cathode indicating a cationic protein. This band disappeared during destaining of the gels, and was likely to coincide with (g), (Figure 17). It suggests a protein with isoelectric point greater than 8.9, the gel pH, hence a mobility towards the cathode.

Slots 6-9 in Plates 1 and 2 contained GCM samples. Less variability was observed in their main casein bands apart from the sample in slot 9 where the  $\beta$ -casein band lost color intensity during destaining. Minor bands were observed among the GCM samples but were fewer when compared with the PCM. The elution profile of the casein fractions of a GCM sample (Figure 14) was consistent with previous finding for normal milk (2) in which low peak areas were observed for para- $\kappa$ - plus  $\gamma$ -caseins. The other GCM samples had elution profiles consistent with Figure 14. Most of the GCM samples were mid-lactation milk. The pH of the samples were on the average near 6.6. Less indigenous milk proteinase activity has been observed in mid-lactation milk (10), and casein composition in mid-lactation tends to be more stable than in early or late lactation milk (3).

The electrophoretic distribution of skim milk proteins is illustrated in Plate 3. The slots contained samples from the same cows as in the two previous plates. The distribution of the major and minor casein bands were consistent with Plates 2 and 3. Thus the PCM samples (Slots 1-5) showed more variability in their  $\beta$ -casein bands and had more minor bands than the GCM (slots 6-9). The gel which showed the electrophoretic distribution of the whey protein bands was not shown because there was little variation in their protein distribution apart from the non-coagulating sample which had an intensely colored  $\beta$ -lactoglobulin band. Little variation in the whey protein bands agrees with the observations of Reimerdes and Herlitz (17), who reported a very slight increase in whey protein of milk that was stored at 4°C for 100 h and was then coagulated with rennet.

#### Technological implications

There was a trend among the PCM samples; the milk shared some characteristics with mastitis milk, in agreement with the findings of Davies and Law (3), who indicated that late lactation milk and mastitis milk had similar properties. They established that a high positive correlation ( $r = .885$ ) existed between milk sodium and  $\gamma$ -casein, a negative correlation ( $r = -.75$ ) between sodium and  $\beta$ -casein, and also  $r = -.51$  between sodium and  $\alpha_s$ -casein existed. Late lactation milk is low in lactose and high in sodium, and such milk is comparatively poor in  $\beta$ - and  $\alpha_{s2}$ - and rich in  $\gamma$ - and para- $\kappa$ -casein (2,3).

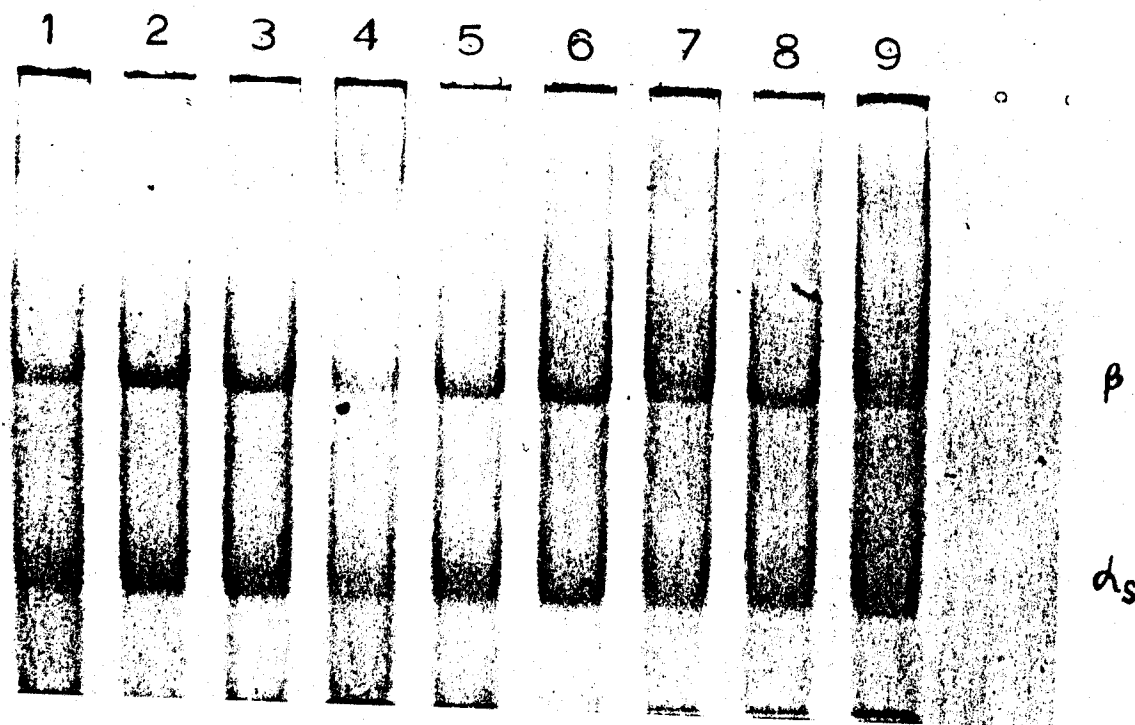


Plate 3. Polyacrylamide electrophoresis in urea gels of skim milk from individual Holstein cows. Slots 1-5, and 6-9 contained poor-, and good chymosin-coagulating milk samples respectively.

The PCM samples contained some blood constituents which might have reacted with the caseins and converted them to products which did not participate in curd structure formation but may have been trapped in the curd. Such curds were very weak and unsuitable for cheese production. Poor curd formation is not only attributed to the presence of these minor casein components in significantly large quantities, but also to increased content of monovalent cations (eg. sodium) as common in late lactation milk. Monovalent cations do not favor the secondary stage of milk clotting. They do not enhance rapid aggregation of casein micelles after chymosin cleavage of the phe 105 - met 106 bond (6).

#### Effect on the kinetics of milk coagulation

The para- $\kappa$ -casein-like substance observed in significant quantities in PCM samples should have initiated aggregation of casein micelles, and should have caused better coagulation, but this never happened. Since para- $\kappa$ - and  $\gamma$ -caseins were eluted under the same peak, the proportion of each variant under the peak could not be ascertained but  $\gamma$ -caseins content are expected to be larger.  $\kappa$ -casein, which is the substrate that is converted to para- $\kappa$ -casein during chymosin cleavage generally has a lower content than the other major casein variants. Thus, stoichiometrically, the content of its cleavage product should be proportional to its original substrate. In addition, if para- $\kappa$ -casein was produced by plasmin cleavage, micelle aggregation might not

have occurred. This is because  $\beta$ -casein, which also participates in micelle aggregation, would be cleaved resulting in the loss of hydrophobicity to the new peptide products. Thus  $\beta$ -casein would remain in solution. Some hydrophobic bitter peptides have been implicated to be the products of  $\beta$ - and  $\alpha_s$ -caseins cleavage (20). The possibility that bitterness in cheese is associated with these cleavage products of  $\beta$ - and  $\alpha_s$ -caseins in late lactation milk should be considered.

## SUMMARY

Five good chymosin-coagulating, and four poor chymosin-coagulating individual cow milk samples were analyzed for casein composition using hydroxyapatite and polyacrylamide gel electrophoresis to establish possible relationships between casein variants and differences in coagulation properties.

The samples exhibited a wide variation in casein composition. The poor chymosin-coagulating milk had higher content of  $\gamma$ - and para- $\kappa$ -caseins, lower  $\kappa$ - and  $\beta$ -caseins than the good-coagulating milks. A poor-coagulating milk sample had an additional casein variant, tentatively identified as  $\lambda$ -casein. Substantial peaks of unidentified minor protein were apparent in a poor-coagulating milk sample which coagulated early but the coagulum did not firm in 30 min. Less variability was observed in the  $\alpha_s$ -casein of all the samples studied.

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# Rapid Estimation of Native Whey Protein in Dry Whey to Predict Loaf Volume of Bread

July 25, 1984

by Ronald B. Malouf

In an effort to predict functional properties of whey, modifications of the Harland-Ashworth test have been made by Jorge Reyes (1976), Reyad Aboumahmoud (1981), and Ron Malouf (1984). Malouf found that 46% of the variation in bread loaf volume could be predicted by using the test. In Figure 1, this weak correlation shows that the extent of denaturation of whey protein has little effect upon loaf volume when whey is added to bread. Up to 8% whey powder was added to the bread without greatly reducing the loaf volume. The data in Figures 1 and 2 is from loaves with optimized potassium bromate (oxidant), water in the formula, and mixing time. The amount of lactose in the added whey was probably the most damaging factor to loaf volume.

Figure 2 shows that the actual temperature of the heat treatment given to the whey might be helpful in predicting loaf volume.

It is hoped that the test can be used to make better predictions of other functional properties of whey.

A detailed description follows of how to perform the test. Casein and denatured whey protein are precipitated with NaCl, and absorbance at 280 nanometers is used to estimate the amount of native whey protein remaining in solution. There is the possibility of running the test in as little as 17 minutes.

## Equipment Required

UV spectrophotometer, preferably capable of reading A 279nm with a 1nm slitwidth;

Quartz cuvettes: 4 for a double beam instrument, 2 for a single beam instrument;

Analytical balance;  
Water baths for 37C and 16C (and thermometers);  
Pipettor for 1000 uL, preferably a Rainin/Gilson P-1000  
Pipetman for the best precision;  
Pipetter pump (10 mL, for trichloroacetic acid solution);  
Ring stand and clamps to hold a 10 mL syringe and a small  
tin can;  
Cylindrical plastic container and metal weights, such as  
steel shot, to weigh a total of 403 g, to give 3-4 psig  
in a 10 mL syringe;  
Small tin can to use as a guide for the 403 g weight;  
Optional: pressure gauge (0-15 psi) and fittings to monitor  
pressure generated in the syringe for filtration.

**Glassware (borosilicate glass):**

Test tubes and vials with Teflon-lined screw caps:  
25 x 150 mm (1 per sample),  
15 x 125 mm (usually 3 per sample),  
4 mL vial (usually 2 per sample);  
Volumetric pipets: one each: 25 mL and 10 mL  
(see text for recommended details);  
Reagent containers for storing trichloroacetic acid solution,  
water, and buffer.

**Materials used:**

Millex-GV (Millipore) filter units (2 per sample);  
alternatively, hydrophillic Durapore (Millipore) membrane  
filters can be used in a Swinnex-25 (Millipore) holder;  
Syringe, polypropylene, 10 mL (2 per sample);  
Reagents: (reagent grade) trichloroacetic acid, mono- and  
dibasic sodium phosphate, NaCl, and distilled water;  
Tips for pipettor (recommended: Rainin RT-200);  
Recommended: self sticking labels for the screw caps,  
1/2 inch diameter.

**Preparation:**

Clean and dry glassware;  
Prepare 3 reagents:  
distilled water, store refrigerated;  
buffer: 0.1000 M sodium phosphate (0.0500 M monobasic and  
0.0500 M dibasic, about pH 6.8), store refrigerated;  
trichloroacetic acid (TCA): 13.20% weight/volume;  
Weigh and pipet reagents; it is recommended that all  
pipetting be done at 22C; for each sample:  
Weigh 10.00 g NaCl and store it in a 15 x 125 mm screw cap  
test tube;  
Pipet 25.00 mL of buffer into a 25 x 150 mm screw cap  
test tube; store refrigerated;  
Pipet 10.00 mL of distilled water into a 15 x 125 mm screw  
cap test tube; store refrigerated;  
Pipet 10.00 mL of TCA solution into a 15 x 125 mm screw  
cap test tube, using a pipetter pump.

**Running the test;** a room temperature of 22C is recommended;  
Bring water baths to 37C and 16C;

Warm buffer and water reagent tubes to 22C;  
 Warm up spectrophotometer;  
 Weigh 1.000 g whey powder and quantitatively transfer it into a tube containing buffer;  
**Put the whey into solution** by gently inverting the capped tube for about 60 sec or until the powder appears to be dissolved. Put the tube into the 37C water bath for 30 min, and mix every 5 minutes by gently inverting the tube three times; **avoid vigorous mixing** that generates foam and theoretically could denature some of the protein;  
**Saturate with NaCl:** pour 10 g of NaCl into the whey-and-buffer solution, close the cap tightly, completely **immerse** the tube in the 37C water bath, and **continuously mix** for 5 min by gently inverting. The important point is to saturate the solution with NaCl at 37C; the test results will not be significantly changed if the saturation is done more slowly or with different agitation, or if the solution remains at 37C for up to 60 minutes;  
**Cool** to approximately 22C by putting the tube in the 16C water bath, leaving it upright and still for 120 sec, with some agitation of the water bath water;  
**Filter** the solution. Mix the tube by inverting three times. With the syringe outlet loosely covered, pour about 10 mL into the open end of the syringe which is mounted on the ring stand with a clamp. Insert the syringe plunger, allowing some solution to be discharged to waste. Attach the filter unit to the syringe; gently depress the plunger about 2 mm and release it. Wait 60 sec before applying pressure to the plunger by resting the 403 g weight on the plunger; the tin can, mounted above the syringe with a clamp, serves as a guide for the descending weight. **Discard the first 12 drops** of filtrate, then collect about 3 mL of filtrate in a 4 mL vial; (see discussion below about the volume of filtrate to collect);  
**Pipet 1.00 mL of filtrate into 10.00 mL of TCA** after inverting the filtrate vial 3 times. Do not prerinse the pipet tip. Discharge the pipettor at about 2 drops per sec to minimize retention of the viscous filtrate in the tip. Do not rinse the tip in the TCA. Mix the tube by inverting 3 times, then set it aside;  
 Similarly **pipet 1.00 mL of filtrate into 10.00 mL of** distilled water, using a new pipet tip. Mix the water dilution by inverting three times;  
**Read the absorbance of the water dilution** immediately if desired; refrigerate the dilution if reading will be delayed long enough to allow growth of microorganisms. The blank for this reading is a solution prepared identically except no whey is added to the buffer. **Solutions should be about 22C when taking readings**  
**Filter the TCA solution.** Attach the filter unit to the syringe before pouring the TCA solution into the open end of the syringe; also, a beaker should be in position to catch waste filtrate before filling the syringe. Fill the syringe, insert the plunger, and apply the 403 g weight immediately. Discard the first 6 1/2 mL of

filtrate and collect the last 3 1/2 mL of filtrate in a 4 mL vial.

**Read the absorbance of the TCA filtrate.** The blank for this reading is a solution prepared identically except no whey is added to the buffer;

Subtract the TCA reading from the water dilution reading, giving the final reading.

#### Comments

Use of the Swinnex-25 filter holder might have some advantages over use of the Millex-GV filter unit. Blank solutions filtered through Millex-GV units have increased absorbance at 279 nm. The first 2 mL of the first filtrate has A 279 nm that is 0.016 higher than the next 2mL; this error is mostly eliminated by the 1-to-11 dilution. The first 3 mL of the TCA filtrate has A 279 nm that is 0.018 higher than the last 3 mL of filtrate from a starting volume of 11 mL; this error might possibly be eliminated by flushing Durapore membrane filters with distilled water, drying the filters, and using them in Swinnex-25 holders. Water might be able to extract from the membrane the same substances that are extracted by the TCA solution. With deionized water, the first 2mL through the Millex-GV unit has A 279 nm 0.022 higher than the next 2 mL of filtrate, which is similar to the difference with TCA filtrates. (The chemical compatibility of the silicone rubber o-ring of the Swinnex-25 is probably adequate for a short time; however, physical changes were visible after 5 days at room temperature in a typical TCA solution.)

If binding to the Durapore membrane filter by proteins and protein fragments is insignificant, which is likely, only about 0.5 mL of the first filtrate would be needed. The first portion of filtrate could be used because it would have the same concentration of protein and nonprotein substances as the later filtrate. This would take less than

one min to filter, instead of up to 6 min to collect 2 mL from some samples.

Instead of pipetting 1 mL into 10 mL, 200 uL portions could be pipetted into 2 mL. None of the TCA filtrate would need to be discarded, and the 0.5 mL lost in the Swinnex-25 holder leaves 1.7 mL of filtrate, enough to read in a regular cuvette. The pipetting errors with the smaller volumes are only slightly larger than the pipetting errors with the larger volumes.

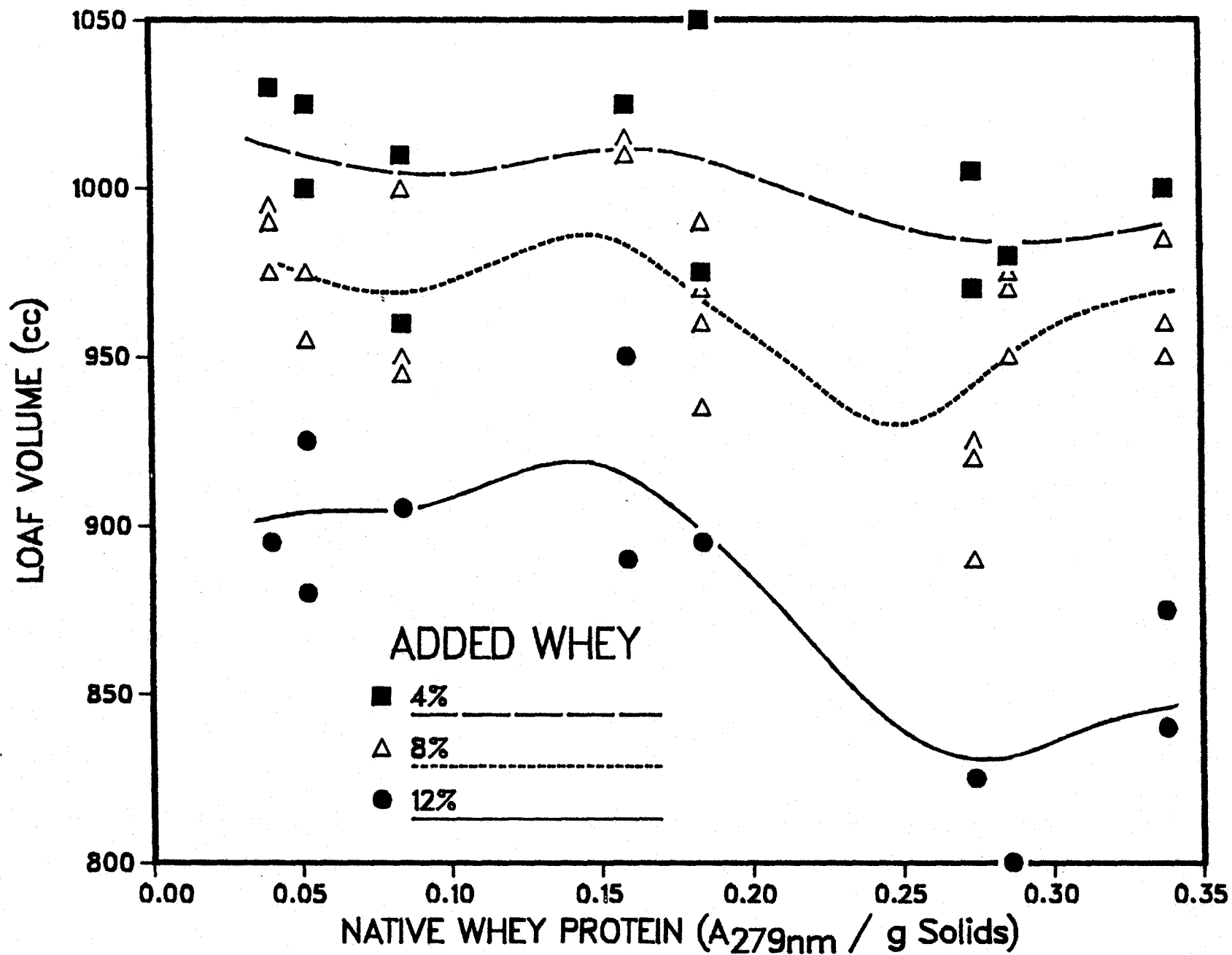
If extra precision is needed from the **volumetric pipets**, the portion of the pipet with the calibration mark can be replaced (by a glassblower) with tubing that has a smaller diameter. This allows more precise use of the mark, but requires recalibration of the pipet, and makes cleaning of the pipet slower.

The time used for the TCA precipitation, from 1 to 60 min, does not change the result. Also, agitation of the TCA solution during precipitation does not change the result.

Allowing the first precipitation solution to stand undisturbed, without any agitation, reduces its membrane-clogging nature. The solution can be left at room temperature for at least 24 minutes, and probably much longer, before filtration, without changing the result.

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Figure 1. Correlation between native whey protein and loaf volume.



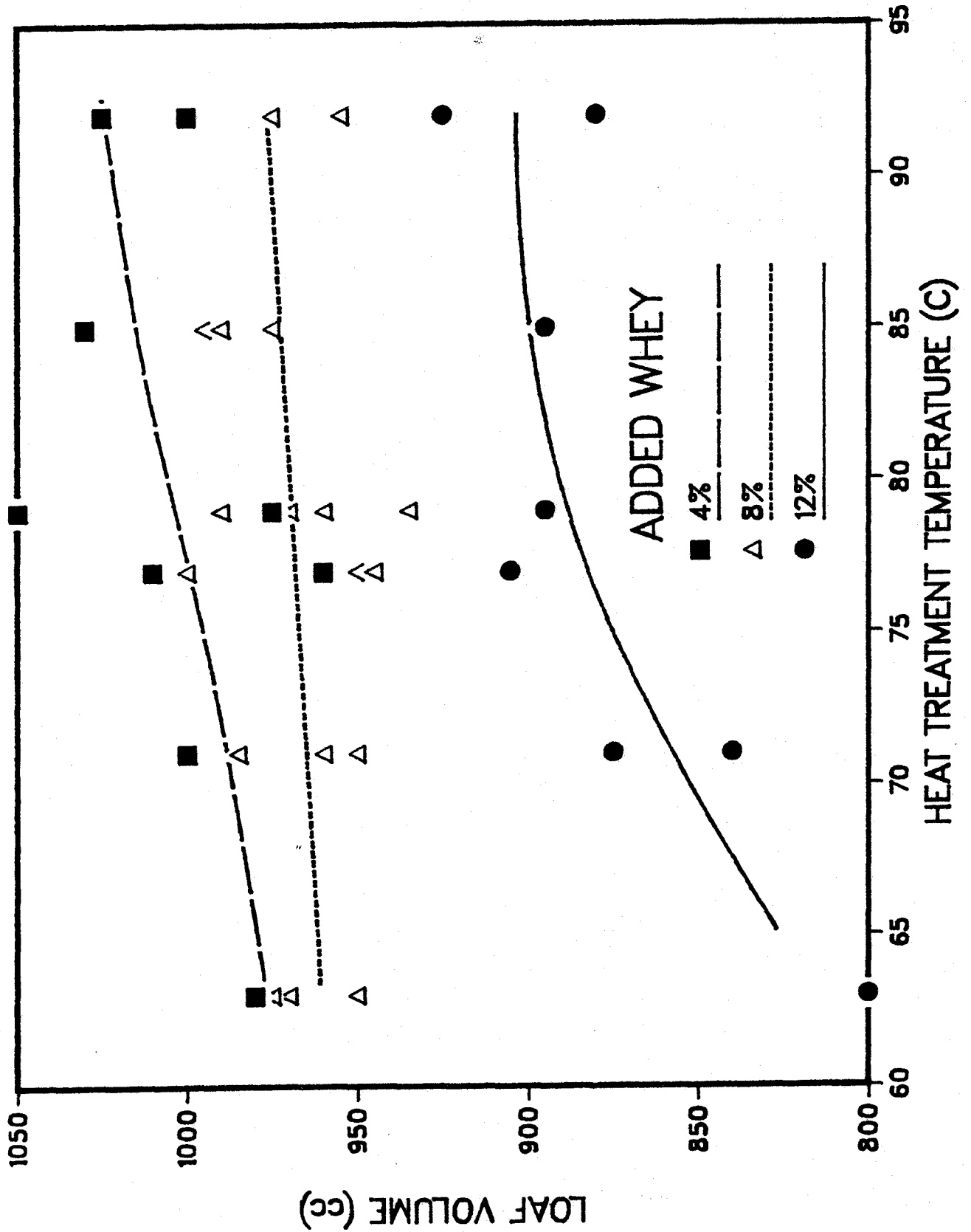


Figure 2. Correlation between heat treatment temperature and loaf volume.

Production of a High Moisture White Cheese  
From Ultrafiltered Whole Milk  
S.M.K. Anis and C.A. Ernstrom

Domati cheese is the major soft cheese produced in Egypt, and is of great importance to the Egyptian consumer. It is a "pickled cheese" since it depends on a high salt content to retard spoilage. It normally contains 48-58% moisture, 20-26% fat and about 5% salt. Some of the Egyptian domati is consumed fresh, although some is allowed to cure for several months to develop a sharp flavor.

The traditional method for making domati cheese requires the addition of 5 to 15 percent salt to a mixture of cows and buffalo milk. Starter may or may not be added prior to setting with rennet. The curd is ladled into special containers or cheese cloth bags and allowed to drain for 1-3 days before it is ready for consumption as fresh cheese.

Mahmoud and Kosikowski have reported considerable yield advantages when domati cheese is made from ultrafiltered skim milk recombined with cream.

The objective of this study was to evaluate the manufacture of domati cheese from ultrafiltered whole milk and ultrafiltered reconstituted skim milk and cream and compare the composition and quality with domati cheese made by the traditional process.

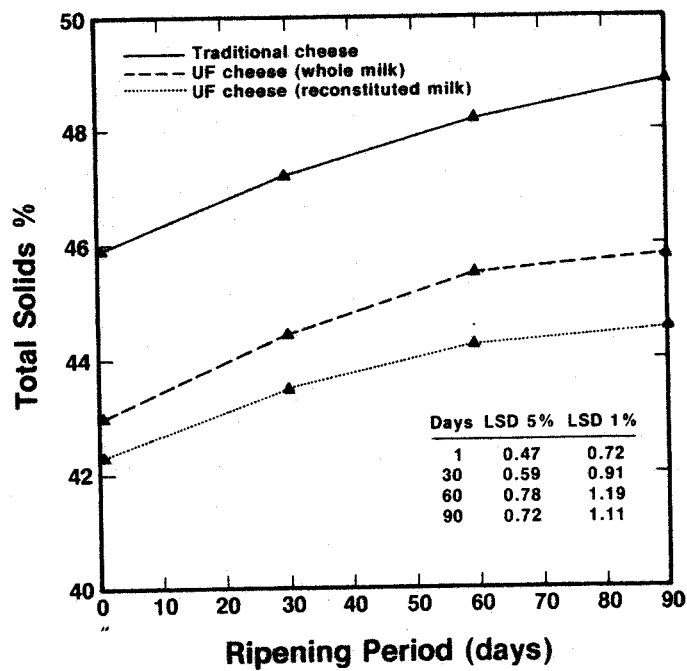
Fresh whole cows milk containing 3.6% fat and 13.0% total solids and reconstituted low heat nonfat dry milk and cream containing 3.7% fat and 12.8% total solids were separately pasteurized at 63°C for 30 min and ultrafiltered at 54°C in 5M<sup>2</sup> Abcor spiral wound ultrafiltration module. Ultrafiltration was to a 5X concentration. The 5X retentates containing 20% fat and 40% total solids were adjusted to 37°C and inoculated with .5% each of S. thermophilus and L. bulgaricus culture.



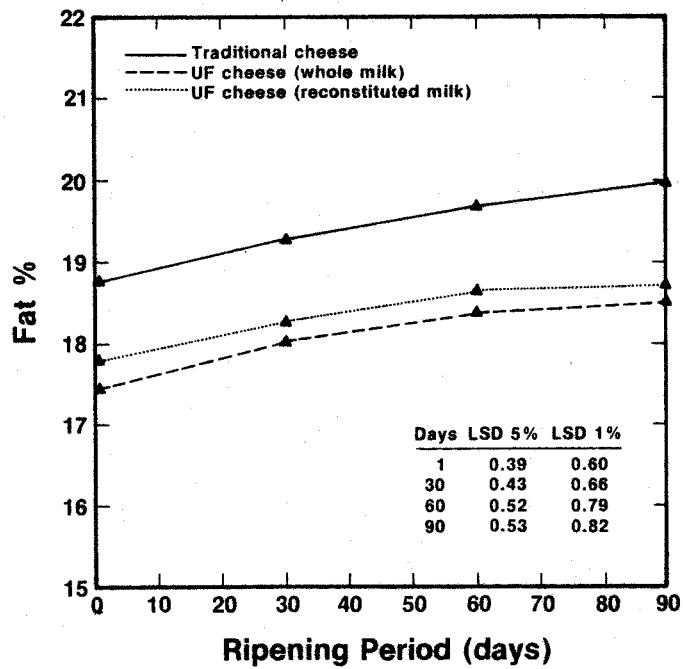
Dry salt was added to a level of 5% in the retentate after which 4.4 ml single strength calf rennet per 100 kg retentate was added. The retentate was then measured in 195 g portions into enameled cans 8.5 cm in diameter by 4.5 cm high. The cans were then sealed and left at 22°C for 90 days.

Domati cheese made by the traditional process from pasteurized cows milk containing 3.75% fat and 13.1% total solids served as the control. The control cheese was made by adding 5% salt to the milk along with 1% of a 50-50 mixture of L. Bulgaricus and S. thermophilus culture. It was set for 2.5 hours with 100 ml single strength rennet per 1000 lbs milk. The curd was then ladled into frames lined with cheese cloth and allowed to drain for 2 days. It was then cut into 4x4x2 cm blocks, placed in cans and covered with pasteurized whey containing 5% salt. The control cheese was stored for 90 days at 22°C. Each treatment was replicated 4 times. Cheese samples were analyzed for total solids, fat, pH, total volatile fatty acids and formol ripening index. The cheese also was judged by a 7 member panel for flavor and degree of mealiness on a linear scale of 1 to 5; 1 being highly objectionable and 5 being highly desirable.

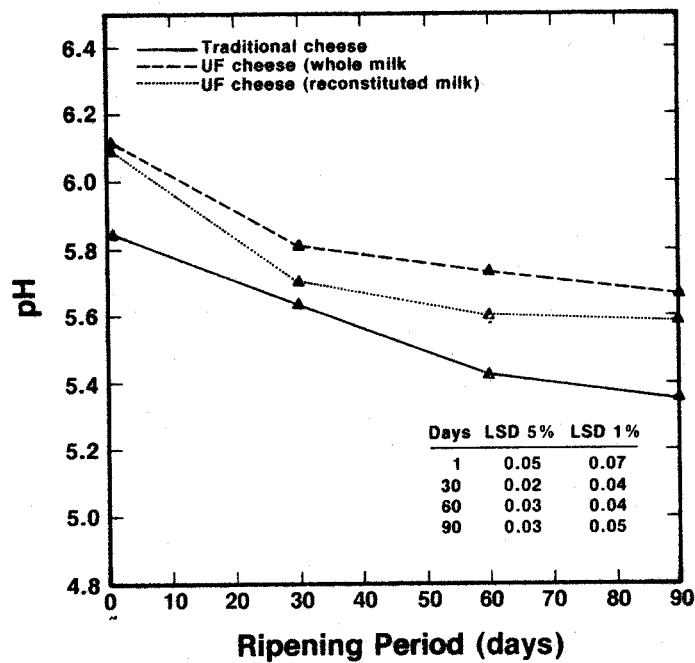
Slide 1 illustrates the changes in total solids of the cheese during storage. All three cheese underwent syneresis during storage. However, both samples made with ultrafiltered milk maintained higher moisture levels (lower solids in the curd) throughout the entire ripening period. Changes in the fat content during storage followed the same general pattern as the total solids (slide 2). The salt concentration (slide 3) retarded the production of acid in all samples, but since the salt in the water phase was lower in the conventional



Slide 1. Total solids during ripening of domiati cheese made from milk by the traditional method, from ultrafiltered whole milk and from ultrafiltered reconstituted milk.



Slide 2. Changes in fat content during ripening of domiati cheese made from milk by the traditional method, from ultrafiltered whole milk and from ultrafiltered reconstituted milk.



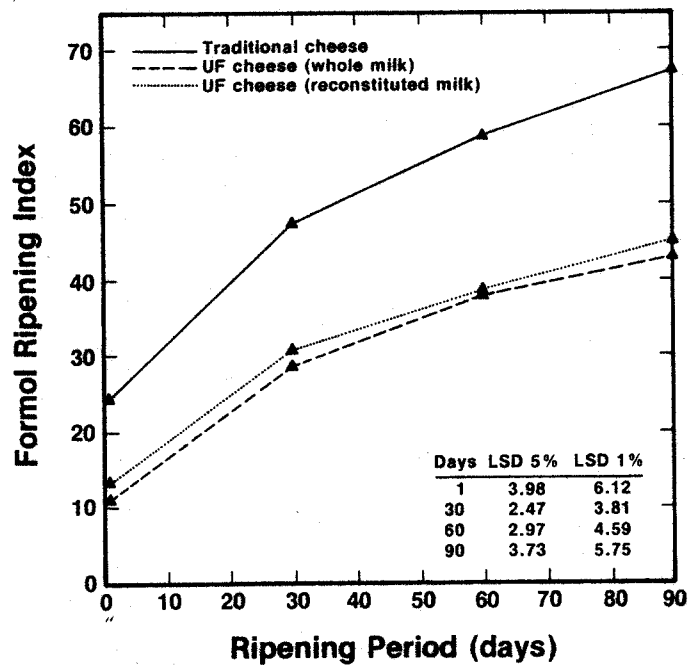
Slide 3. pH changes during the ripening of domiati cheese made from milk by the traditional method, from ultrafiltered whole milk, and from ultrafiltered reconstituted milk.

domiati the pH change was somewhat more rapid than in the UF cheese. In no case did the pH reach low enough levels to be considered normal for low salt cheese varieties. Slide 4 shows changes in the formol ripening index which illustrates a more rapid proteolysis in the conventional than in the UF cheese.

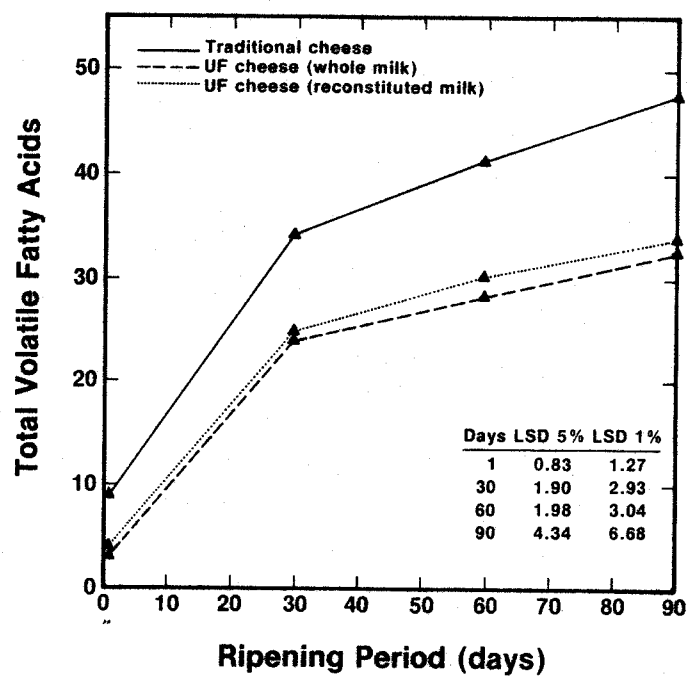
The same relationship is shown in slide 5 for changes in volatile fatty acid production.

Yield advantages due to ultrafiltration are illustrated in slide 6 where percentages of original milk solids (including salt) fat, and nitrogen recovered in the freshly made cheese are shown. Fat recovery in both ultrafiltered samples was 100%, and 95% in the traditional product. Nitrogen recovery was nearly 98% in ultrafiltered samples as opposed to 75% in the traditional cheese. Both of these constituents are reflected in a higher recovery of total solids in the ultrafiltered samples. Average flavor and body scores for the ultrafiltered samples were significantly higher than for the traditional cheese (slide 7). Differences between cheese made from ultrafiltered whole milk and ultrafiltered reconstituted milk were not significantly different. All the samples were criticized for high salt which is not a defect in traditional domiati cheese. The most frequent flavor criticism of the U.F. samples was lack of acid. In general the U.F. cheese was firmer and smoother in texture than cheese made by the conventional method.

The manufacture of domiati cheese from 5X ultrafiltered whole milk (either regular or reconstituted) resulted in a product as good or better than the traditional cheese. The UF product had better moisture retention and much better recovery of fat, protein and milk solids than the traditional product. Protein breakdown and production of volatile free fatty acids was slower than in the traditional product.



Slide 4. Changes in formol ripening index during curing of domiati cheese made from milk by the traditional method, from ultrafiltered whole milk and from ultrafiltered reconstituted milk.



Slide 5. Changes in volatile fatty acid content during ripening of domiati cheese made from milk by the traditional method and from ultrafiltered whole milk and ultrafiltered reconstituted milk.

Slide 6: Recovery of milk solids in fresh Domiati cheese made from ultrafiltered whole milk (UFWM) and ultrafiltered reconstituted milk (UFRM) compared to traditionally made cheese, (control).

Milk Constitutents	Control		UFWM		UFRM	
	%	SD	%	SD	%	SD
Total solids*	47.42	± .19	63.77	± .22	63.04	± 1.09
Fat	95.06	± .47	100.01	± .09	99.97	± 0.00
Nitrogen	75.17	± 1.03	97.97	± .29	97.95	± .35

\*(including salt).



Slide 7: Flavor and body scores\* of Domiati cheese (1 month old) made from ultrafiltered whole milk (UFWM) and ultrafiltered reconstituted milk (UFRM) compared to the traditionally made cheese (control).

(Average of 7 panelists)

		Control	UFWM	UFRM
Flavor	Av. score	2.86	3.93	4.50
	SD	± 1.07	± .53	± .41
	Comments	yeasty	lacks acid	lacks acid
Body	Av. score	2.43	3.86	4.29
	SD	± .35	± .69	± .76
	Comments	mealy pasty not smooth	rubbery	firm

Scores\*: 1 - highly objectionable  
5 - highly acceptable

An attempt was next made to modify domiati cheese so it might be more acceptable to markets other than those in the Middle East. This would require a reduction in the salt content and a rapid production of acid to a pH of 5.1 to 5.2 but no lower.

Fresh whole cows milk containing 3.7% fat and 12.95% total solids was pasteurized at 63°C for 30 minutes, cooled to 54°C and ultrafiltered until 60% of the original milk weight had been removed as permeate. The retentate was then diafiltered at constant volume by bleeding 54°C deionized water into the supply tank at the same rate permeate was being removed until an amount of water equal to 38.5% of the original milk weight had been added. At that point the diafiltration water was turned off and ultrafiltration was continued to a 5X concentration.

Preliminary experiments in which the retentate was inoculated with lactic culture and set with rennet resulted in cheese with an appropriate pH of 5.15 but which continued to expell whey and was considered very mealy and spongy.

Similar retentate was then divided into five parts and heated for 30 minutes at 65.5, 71.1, 76.6, 82.2 and 87.7°C before cooling to 11°C and inoculating with 1% lactic culture. Retenate representing each heat treatment was divided into two lots. To one lot was added 2.5% salt, renneted (3 ml/100 lb) and sealed in cans. The other lot was renneted and placed in cans with a circle of parchment under the lid. Salt equal to 2.5% of the retentate was placed on the parchment under the lid. This was to delay the absorption of the salt into the cheese until the starter had had a chance to reduce the pH.

All cans were incubated upside down at 22°C for 1 day then turned right side up and incubated for an additional day.

The effect of salting method on rate of pH change is shown in slide 8 for the sample heated to 82.2°C and was typical of all other treatments. Direct salting slowed the pH change although it eventually reached pH 5.1 after 15 days. When salt penetration was retarded by parchment the pH reached 5.4 in one day and 5.15 in 3 days.

Of greatest interest in this study was in the effect of heat treatment of the retentate on the mealiness of the final product. This is illustrated in slide 9. A panel of four judges rated mealiness on a scale of 0 (no mealiness) to 4 (very mealy). As the heat treatment increased the tendency toward mealiness decreased. Furthermore the tendency for whey expulsion also decreased.

It was concluded that a cheese similar to domiati, but with a reduced salt content, controlled pH, and extended shelf life can be made from ultrafiltered whole milk by heating the retentate to at least 82.2°C for 30 min prior to culturing and setting.

A preliminary experiment investigated the effect of heating the milk prior to ultrafiltration rather than heating only the retentate. In this instance it was impossible to reduce the moisture in the retentate below 67%. At that point all permeation stopped.

Slide 8: Effect of salting method of the pH of Domiati cheese made from ultrafiltered whole milk and stored for one month at 22°C.

Storage (Days)	Retentate heat treatment: 82.2°C/30 min			
	D		P	
	pH	SD	pH	SD
0	6.68 ± .02		6.64 ± .01	
1	6.10 ± .04		5.40 ± .02	
2	5.80 ± .08		5.30 ± .04	
3	5.50 ± .06		5.15 ± .04	
15	5.25 ± .04		5.05 ± .02	
30	5.10 ± .05		5.05 ± .02	

D = 2.5% salt dissolved in the retentate before renneting.

P = 2.5% salt dispensed in cheese through parchment paper.

Slide 9: Effect of retentate heat treatment and salting method on the mealiness\* of Domiati cheese made from ultrafiltered whole milk.

	65.5°C/30min.		71.1°C/30min.		76.6°C/30min.		82.2°C/30min.		87.7°C/30min.	
	D	P	D	P	D	P	D	P	D	P
Max.	4	4	4	4	3	3	3	3	2	1
Min.	4	3	3	2	2	1	2	0	0	0
Av. Score	4.0	3.8	3.4	3.0	2.6	2.4	2.4	1.0	1.0	0.2
SD	±0.00	±0.45	±0.55	±0.71	±0.55	±0.89	±0.55	±1.41	±0.71	±0.45

D = 2.5% salt dissolved in the retentate before renneting.

P = 2.5% salt dispensed in cheese through parchment paper.

\*Mealiness score: 4 - very mealy, 0 - no mealiness

## Role of Milk Clotting Enzymes in Cheese Curing

G.H. Majeed and C.A. Ernstrom

Milk clotting enzymes used in cheese making contribute to proteolytic breakdown during cheese curing. However, the evidence is inconclusive as to whether this enzymatic effect is contributory, detrimental or insignificant to overall cheese quality and flavor development.

Much research has suggested an important cheese curing role for milk clotting enzymes, yet only 2-6% of the enzymatic activity added to cheese milk remains in Cheddar cheese after pressing (3), and no activity has been reported in Swiss cheese where a high cooking temperature is employed.

Excessive use of microbial milk clotting proteases as well as chymosin can result in unacceptable bitter flavor in cheese. It has been suggested that proteases from Mucor miehei and Mucor pusillus are used extensively in cheese making only because such a small amount remains in the curd (2-3%) (3). Melachouris and Tuckey and others (4) produced good quality Cheddar cheese from milk coagulated with porcine pepsin which has very poor stability in milk above pH 6.2 (1). The question then arises as to whether porcine pepsin survives the Cheddar cheese making process and remains active in the curd. O'Keeffe, et al. (5) presented electrophoretic evidence of a proteolytic split in  $\alpha_{s1}$ -casein in Cheddar cheese made with porcine pepsin but without starter (acidified with glucono-delta-lactone) and suggested that the adsorption of pepsin to casein makes it less susceptible to denaturation even though it denatured quite easily in phosphate buffer under the same conditions. They concluded that porcine pepsin survived the

cheese making process under normal cheese making conditions and contributed as much as chymosin to cheese proteolysis.

Holmes, et al. (3) reported a procedure for measuring low concentrations of milk clotting enzymes in curd and whey, but found a pH dependent adsorption of chymosin and porcine pepsin to curd. In order to test for enzyme activity it was necessary to release the enzyme from the curd into a water slurry by raising the pH to 6.8. This, of course, resulted in loss of pepsin activity during extraction. Therefore, the absence of pepsin activity they reported in Cheddar cheese curd was inconclusive.

The purpose of this study (slide) is shown in fig. 1.

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#### FIGURE 1

##### OBJECTIVES

1. Modify the Holmes, et al. procedure to enable recovery of porcine pepsin from curd without loss of activity.
2. Determine whether porcine pepsin survives the Cheddar cheese making process.
3. If porcine pepsin does not survive the cheese making process, explain the  $\alpha_{s1}$ -casein decomposition reported by other workers.
4. If porcine pepsin does survive the cheese making process, determine its effect on cheese caseins.

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##### Modification of the diffusion technique

The modification of Holmes, et al. technique for measuring low concentrations of milk clotting enzymes was based upon the assumption that the interaction between the curd and the enzyme is ionic.

Therefore, this interaction could be weakened by using a specific concentration of sodium chloride solution for enzyme extraction at pH 6.0. The original procedure used water at pH 6.8. The best concentration of sodium chloride to enable complete recovery of chymosin and porcine pepsin in the curd, at pH 6.0. was 1M.

(slide) The chymosin and porcine pepsin recovery in freshly coagulated milk is shown in fig. 2. The controls represent the original diffusion procedure. The modified diffusion technique was applied first to chymosin. The average chymosin units recovered of ten replications in whey and curd was  $100.43 \pm 5.5\%$  of the total chymosin added to milk, which is the same as the control in the original procedure. Then the modified procedure was applied to porcine pepsin and we found that the average of five replications of the total chymosin units recovered in whey and curd was  $102 \pm 5.9\%$  of the total porcine pepsin added to the milk. With the original Holmes procedure there was a loss of about 37% of the total porcine pepsin added to the milk. All this loss of activity was due to the extraction of the enzyme from curd at pH 6.8.

#### Survival of porcine pepsin during Cheddar cheese making

The modified diffusion procedure should answer the question; does porcine pepsin survive the Cheddar cheese making process? Cheddar cheese was made in triplicate from pasteurized whole milk set at pH 6.6., 6.4, 6.2 and 6.0. Porcine pepsin was used as a milk coagulant. The residual pepsin in cheese curd after pressing was measured using the modified diffusion technique.

The amount of porcine pepsin retained in Cheddar cheese after pressing depended on the pH of milk at setting as shown in fig. 3.

(slide) When milk was set at pH 6.0, about  $6 \pm 0.3\%$  of the total



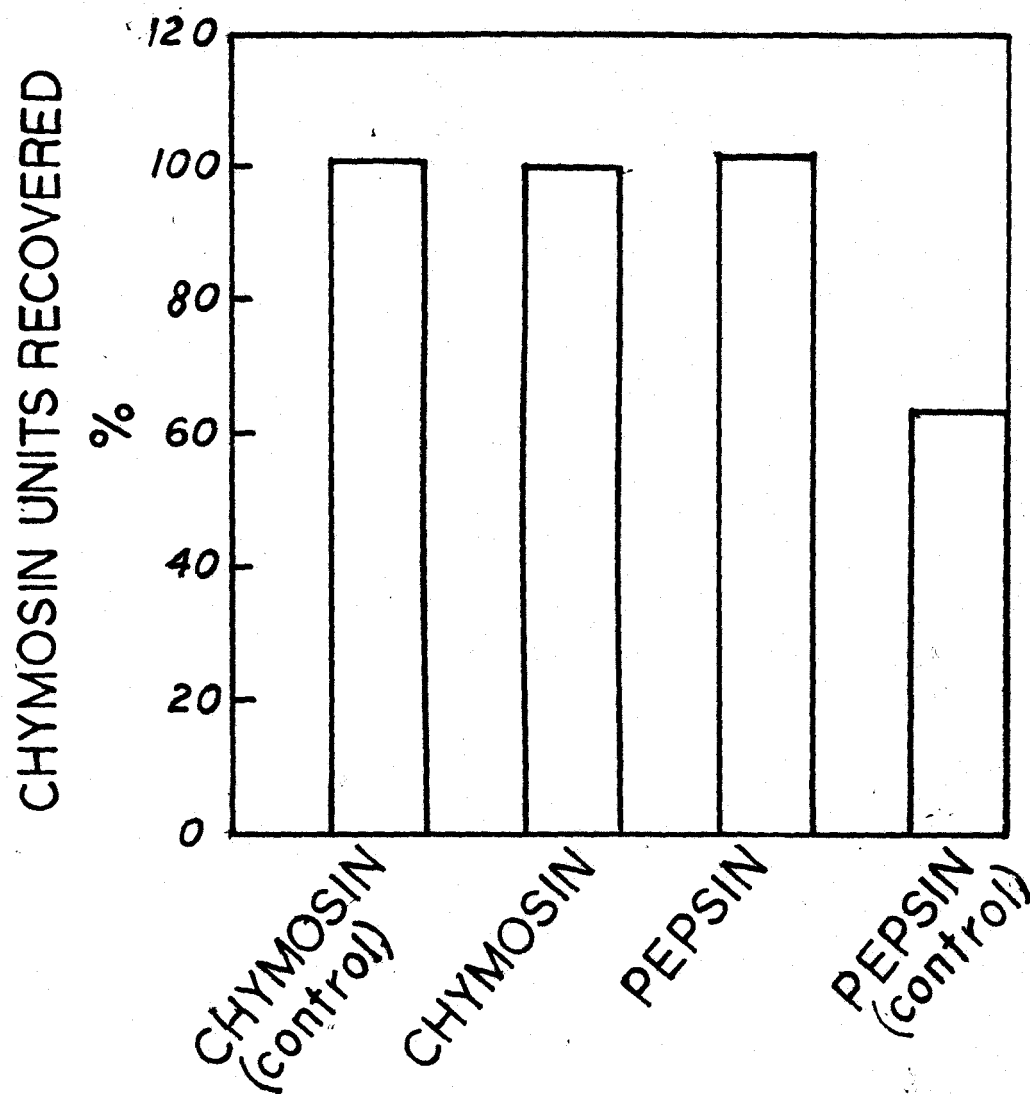


FIG.2 CHYMOSIN AND PEPSIN RECOVERY  
In Freshly Coagulated Milk .

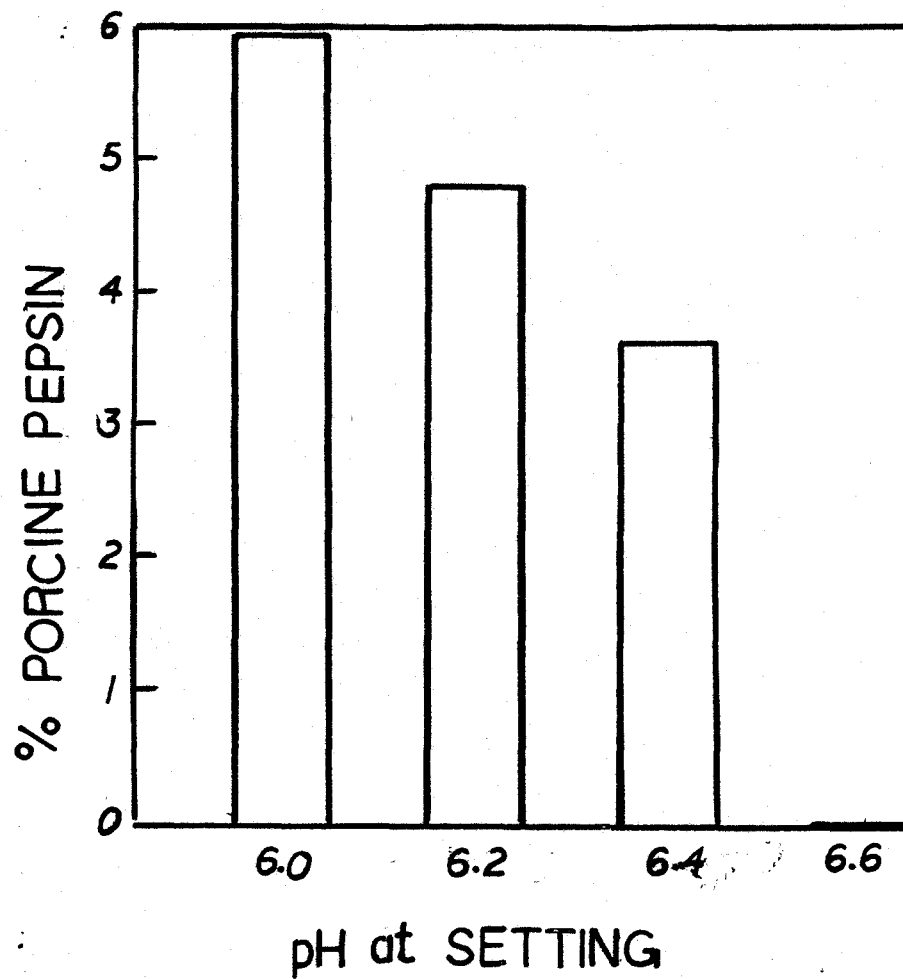


FIG. 3, PORCINE PEPSIN RETAINED IN  
CHEESE CURD AFTER PRESSING

porcine pepsin activity added to cheese milk was recovered in the curd after pressing. At pH 6.2,  $4.8 \pm 0.5\%$  of the total pepsin activity added to cheese milk was recovered in the curd. At pH 6.4,  $3.6 \pm 0.12\%$  of the total porcine pepsin added to cheese milk was recovered in the curd after pressing. At pH 6.6, no porcine pepsin activity was detected in the curd after pressing.

However, it was reported by O'Keeffe, et al. (5) that a breakdown in  $\alpha_{s1}$ -casein occurred even though milk was set at pH 6.6 and 6.65, as shown by their gel electrophoretic patterns, which they attributed to porcine pepsin activity.

We made Cheddar cheese exactly as in O'Keeffe, et al. (5) in which the milk was acidified with lactic acid and glucono delta lactone. Porcine pepsin was used as the milk coagulant at setting pH's of 6.6, 6.4, 6.2 and 6.0. The cheese was examined for  $\alpha_{s1}$ -casein degradation during ripening by means of polyacrylamide urea gel electrophoresis at pH 9.1-9.3 as was done by O'Keeffe, et al. Our gel electrophoretic patterns for cheese at pH 6.6 (fig. 4) agree with those of O'Keeffe, et al. and show no breakdown in  $\alpha_{s1}$ -casein after pressing, but after 10 days ripening very faint degraded bands from  $\alpha_{s1}$ -casein appeared, which increased with increasing time of ripening.

To determine the cause of this degradation, since no porcine pepsin activity was detected in cheese set at pH 6.6, cheese curd free of starter bacteria and milk clotting enzyme was made from ultrafiltered milk acidified to pH 5.2 with hydrochloric acid and glucono-delta-lactone and condensed to 39% moisture at 40°C. (slide) The electrophoretic patterns of the cheese curd which was free of milk clotting enzyme and starter after 6 weeks ripening show the same extent

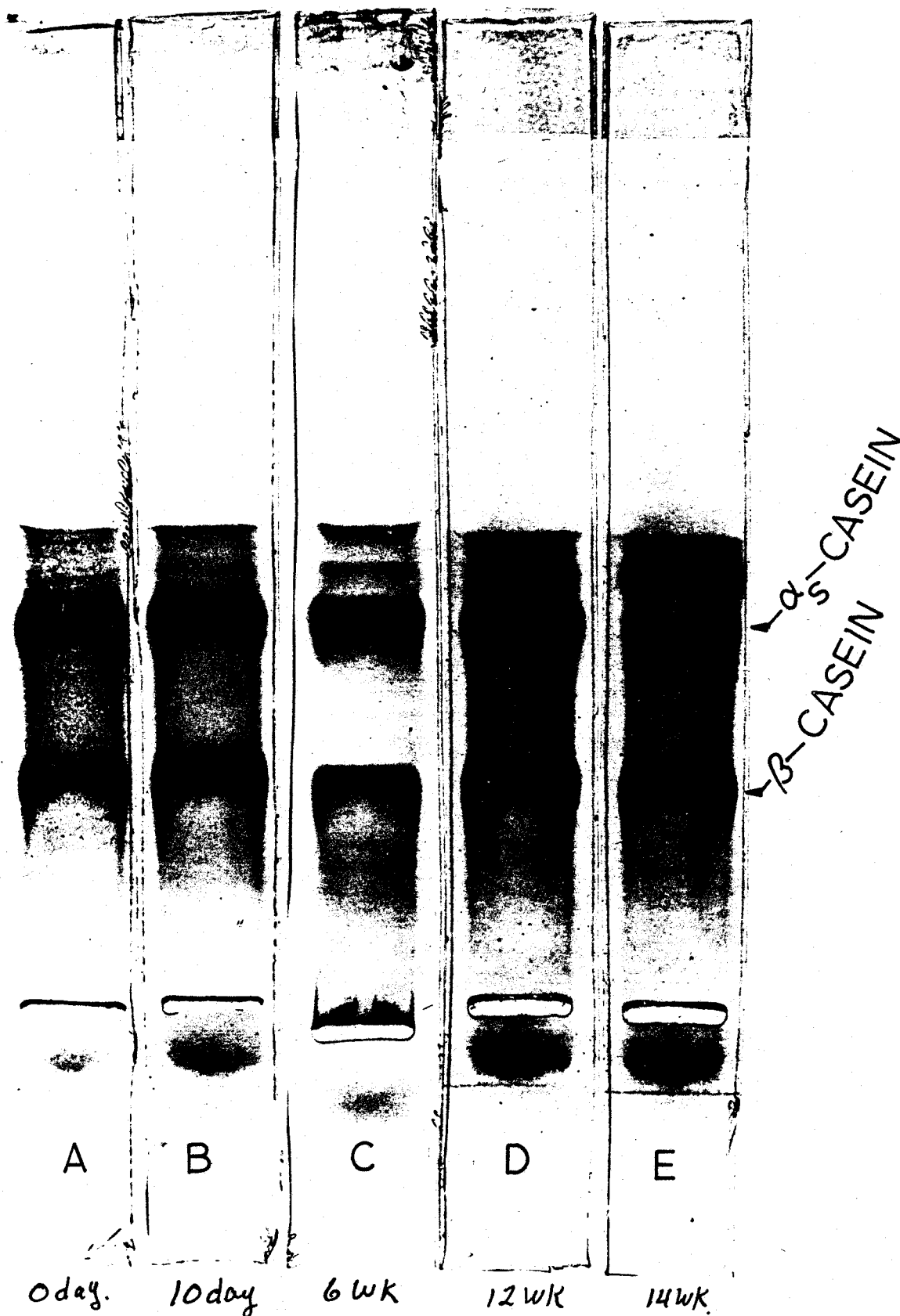


FIG. 4  
Set at pH 6.6.

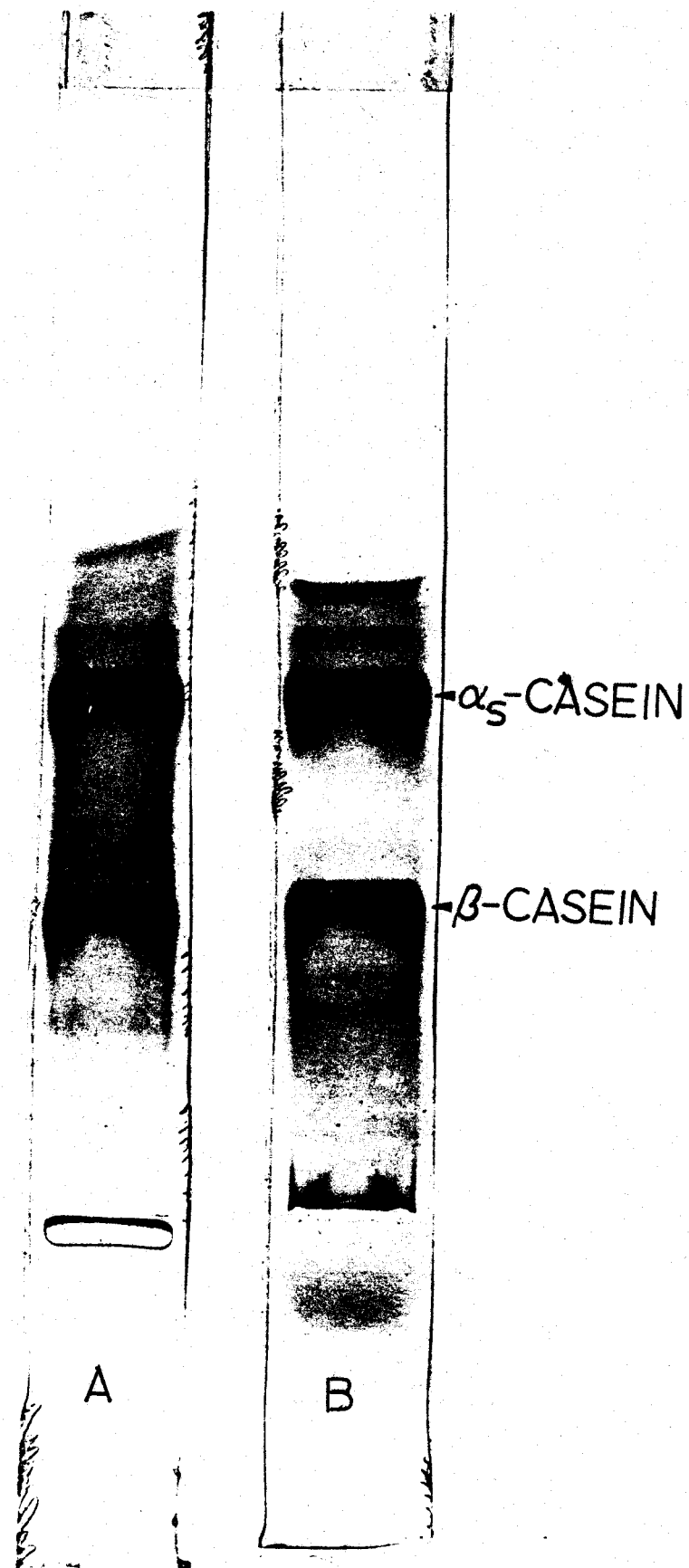


FIG. 5

UF CURD

CHEDDAR

PH 6.6 setting

6 WEEKS

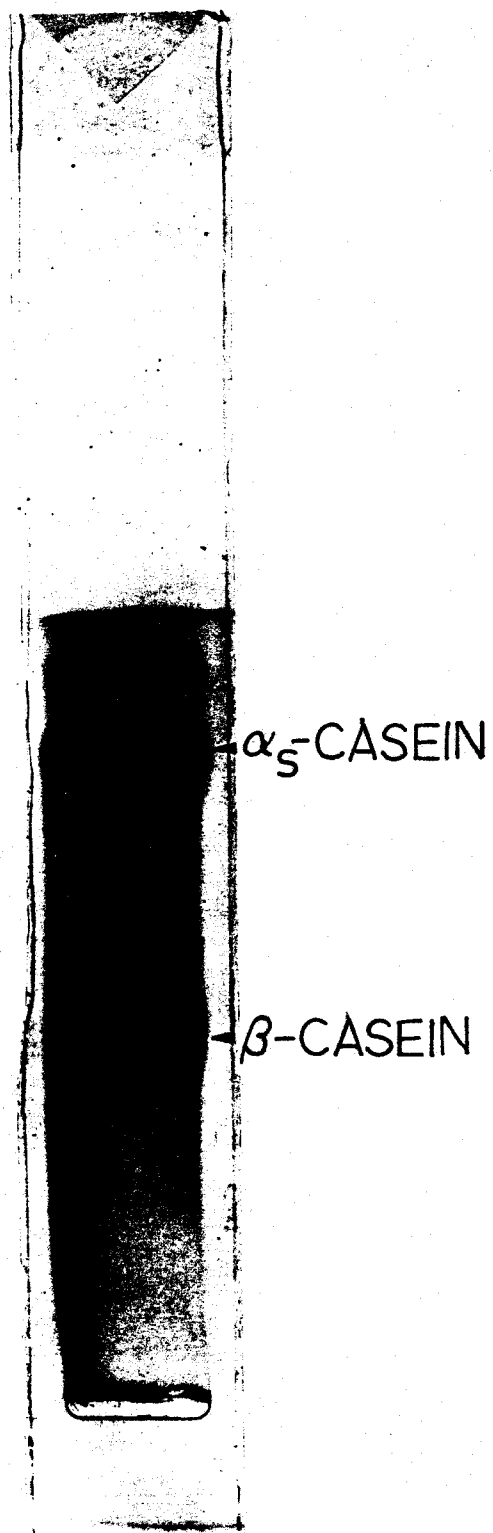


FIG 6. UFCURD 14 WKS OLD

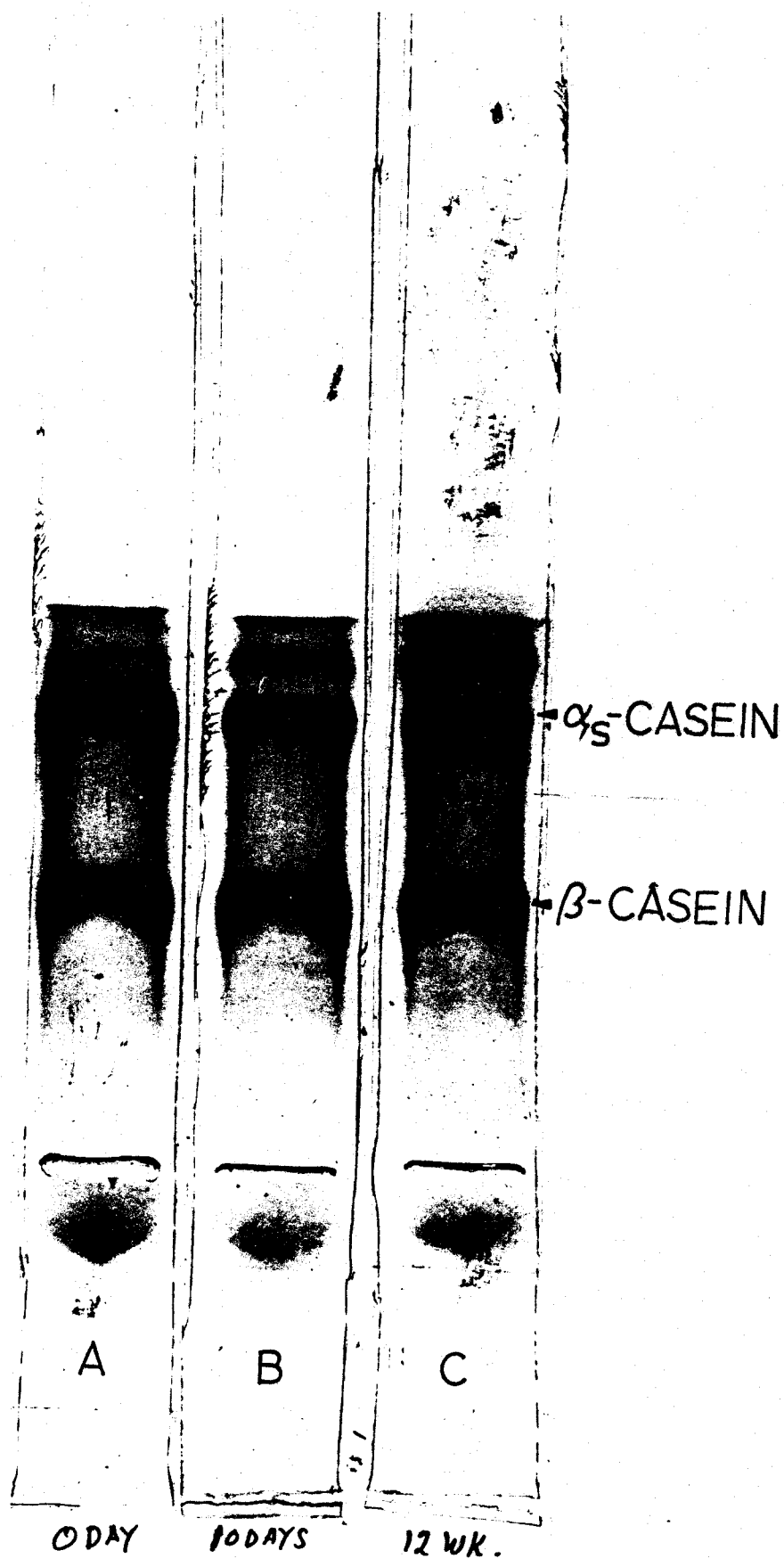


FIG. 7. Cheddar cheese set at pH 6.4 with porcine pepsin.

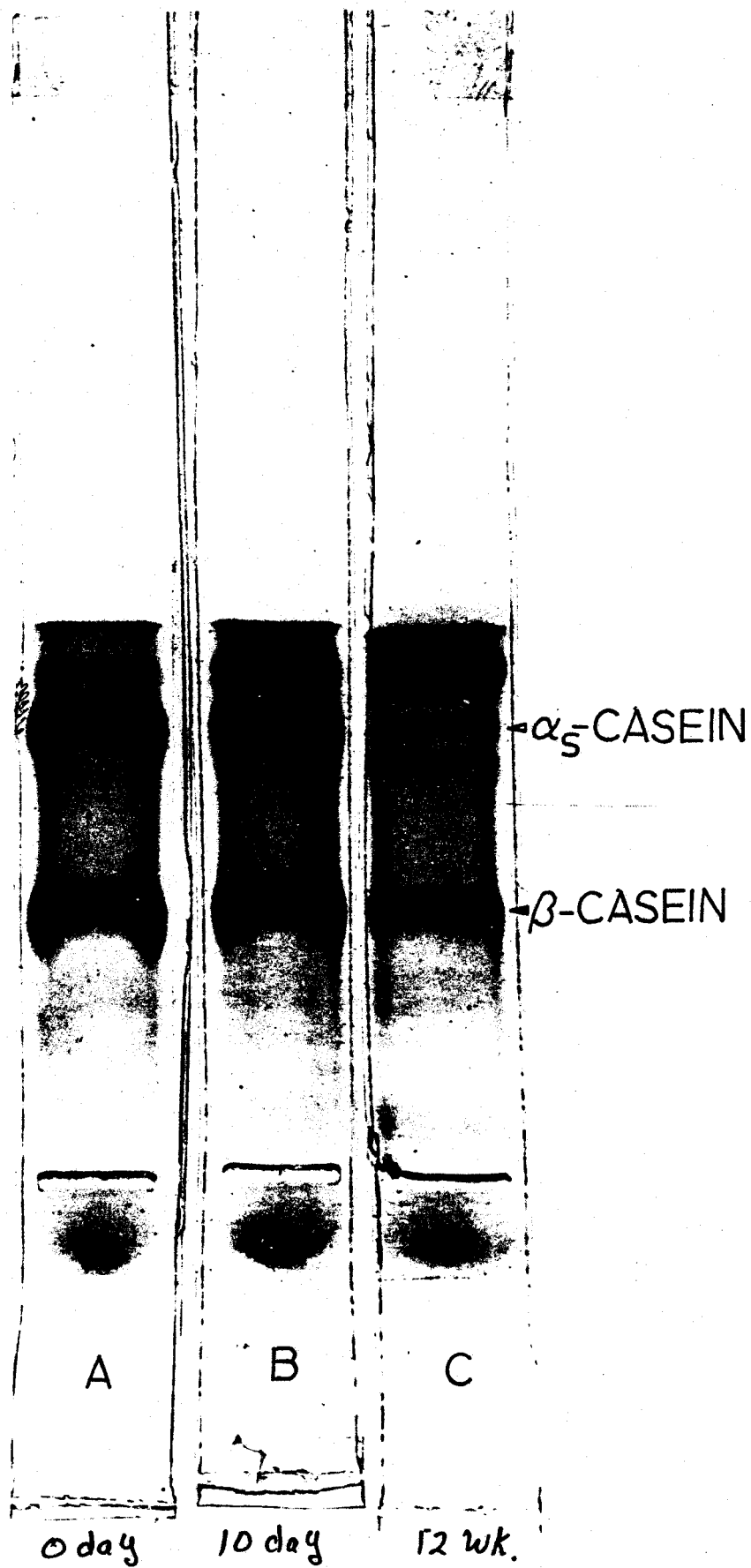


FIG. 8 Cheddar cheese set at PH 6.0 with Pepsin



of degradation as the cheese made with porcine pepsin and set at pH 6.6 (fig. 5). (slide) More degradation of  $\alpha_{s1}$ -casein occurred in the starter-free coagulant-free cheese curd after 14 weeks ripening (fig. 6).

This degradation of  $\alpha_{s1}$ -casein might be caused by milk proteases since the total bacterial count of the cheese curd after 6 weeks ripening was about 7000 cells/g cheese curd. (slide) The gel electrophoretic patterns of cheese set at pH 6.4 show a breakdown in  $\alpha_{s1}$ -casein after pressing which increased after 10 days and 12 weeks ripening because some residual pepsin in cheese curd (fig. 7). Figure 8 represents Cheddar cheese set at pH 6.0. More degradation was evident after pressing and after 10 days and 12 weeks ripening than when the milk was set at pH 6.4 because more porcine pepsin was retained in cheese curd. The conclusions are in fig. 9.

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#### FIGURE 9

#### CONCLUSIONS

1. Porcine pepsin survival during Cheddar cheese making depends upon the pH of milk at setting. The lower the pH the more pepsin was retained in the curd. No pepsin activity was detected in cheese when the pH at setting was 6.6.
  2. Degradation of  $\alpha_{s1}$ -casein during the curing of Cheddar cheese made with porcine pepsin increased as the pH at setting decreased.
  3. Degradation of  $\alpha_{s1}$ -casein occurred even though no porcine pepsin or starter bacteria were present in cheese curd. This may have resulted from action by milk proteases.
  4. When Cheddar cheese was set at pH 6.6, porcine pepsin did not survive the Cheddar cheese making process, therefore, could not contribute to cheese curing.
-

lists mean values for actual yield, moisture adjusted yield and predicted yields using the modified Van Slyke's equation, at different casein/fat ratios. There were no significant differences between predicted yield, moisture adjusted yields and actual yields; indicating that at all casein/fat ratios (.64 to .71), the modified Van Slyke formula (Brown, 1981; Ernstrom, 1980) which assumes a 90% fat recovery predicted cheese yields.

Table 4: Cheese Yield Comparison. Predicted, Actual and Moisture Adjusted.

Casein/Fat Ratio	Percent Yield $\pm$ Standard Deviation		
	Actual	Adjusted <sup>a</sup>	Predicted <sup>b</sup>
0.647 $\pm$ 0.003	11.10 $\pm$ 0.92	11.20 $\pm$ 1.21	11.32 $\pm$ 0.93
0.671 $\pm$ 0.006	11.03 $\pm$ 0.76	10.91 $\pm$ 1.21	11.05 $\pm$ 0.88
0.704 $\pm$ 0.006	10.78 $\pm$ 0.84	10.60 $\pm$ 1.21	10.71 $\pm$ 0.85

<sup>a</sup>=Actual yield adjusted to equal moisture of 37%

<sup>b</sup>=Yield predicted by equation  $(0.9F + 0.78P - 0.1)/(1 - 0.37)$

The large standard deviations shown in table 4 result from the fact that each casein/fat ratio is represented by three different fat and casein percentages in the milk.

When adjusted yield was gauged by amount of cheese produced per unit milkfat, results indicated that at constant casein levels, amount of cheese produced per unit milkfat increased with increasing casein/fat ratio. Conversely, the amount of cheese produced per unit casein decreased as casein/fat ratio was increased.

Mechanization of cheesemaking procedures has led to interests in measuring curd firmness. Olson (1982) has suggested that monitoring

drippings collected during pressing. Average concentration of fat in these drippings was  $12.79 \pm 5.62\%$ . This was about 3.1% of total milk fat and 36% of total fat lost during cheesemaking.

Recovery of Milkfat and Yield of Cheddar Cheese:

Milkfat recovery is of major importance in determining cheese yields. Comparisons of means of milkfat recovered at different casein/fat ratios are shown in Table 3.

Table 3: Average Fat Recovery in Cheddar Cheese (mean, range)

C/F Ratio	% Fat Recovery		
	Mean	Minimum	Maximum
$0.647 \pm 0.003$	91.58	89.56	93.06
$0.671 \pm 0.006$	91.69	89.63	93.00
$0.704 \pm 0.006$	91.46	88.44	92.99

Average fat recovery was 91.58%. At draw, recovery of milk fat was about 94.5%. Additional fat losses which occurred during pressing account for the observed lower fat recoveries than assumed by the Van Slyke equation. The observed ranges of milk fat recovery fell within ranges of 86.49 to 94.32% reported by Van Slyke and Price (1952), but were higher than the range of 83.3 to 87.2% found by Barbano and Sherbon (1980). No significant effect of casein/fat ratio on milk fat recovery was observed within the range of casein/fat of 0.64 to 0.71. Fat losses in whey are therefore independent of the casein/fat ratio within the limits studied.

Observed differences in average moisture content of cheese demonstrated that mathematical adjustment of actual yield to an equal moisture of 37% was necessary for varied yield comparisons. Table 4

### Composition of Cheese:

Compositions of fresh Cheddar cheeses at the different C/F ratios are compared in Table 2.

Table 2: Concentration of constituents in fresh Cheddar cheese (means and standard deviations)

C/F Ratio	Cheese Constituent				
	% Fat	% Protein	% Moisture	% FDM	%MNFS
0.647±0.003	35.4±0.8	23.64±0.13	36.7±1.2	55.9±0.6	56.83±1.21
0.671±0.006	34.5±0.7	24.14±0.87	38.2±0.5	54.8±0.6	56.45±2.18
0.704±0.006	33.2±0.6	24.73±0.35	37.7±1.1	53.2±0.8	56.69±1.59

<sup>a</sup>Moisture in non fatty substance.

An increase in casein/fat ratio resulted in a decrease in cheese fat ( $r = -0.8$ ,  $p < 0.02$ ), a decrease in cheese FDM ( $r = -0.93$ ,  $p < .0005$ ) and an increase in % protein in cheese ( $r = 0.8$ ,  $p < .02$ ). Cheese MNFS were all above the suggested optimum range of 52-54% for Cheddar cheese (Pearce, 1978). They were, however, close to those reported by Olson (1982). A strong negative correlation was observed between milk protein and MNFS ( $r = -0.9$ ,  $p < .001$ ). Increased cheese moisture resulted in increased MNFS, however, variations in MNFS could not be related to casein/fat ratios because of variations in cheese moisture.

### Whey Composition:

Cheese whey, collected at the point of draw was  $88.2 \pm 0.8\%$  of milk weight whereas whey drippings from press formed about 1% of milk. Fat concentration in whey collected at draw averaged  $0.25 \pm 0.03\%$ . This formed about 5.5% of total milk fat and about 64% of fat losses during cheese manufacture. There were large variations in fat content of whey

samples were analyzed for total protein by the AOAC method (16.200) except that sample size was reduced to 0.5g and like milk samples, 2g  $\text{Na}_2\text{SO}_4$  was used as digester with 2 ml of 10%  $\text{HgSO}_4$  as catalyst. The titrating acid was 0.0554 N HCl. A nitrogen conversion factor of 6.38 was used in calculating the content of protein and its fractions. All nitrogen fractions were determined using the Kjeltac Auto System Analyses.

Cheese moisture was determined by the AOAC oven method with slight modification in which 50 ml beakers were used instead of moisture dishes to prevent splashing of cheese solids during drying moisture was taken as weight loss after drying 2-3g of cheese in a forced air oven at 100C for 16h.

### RESULTS AND DISCUSSION

Composition of Cheese Milk: Variations in protein, casein and fat levels of cheese milk are shown in Table 1. For each lot, protein and casein were constant while fat levels varied. Three protein levels were used, and casein/fat (C/F) ratios were between 0.64 and 0.71.

Table 1: Average Protein, Casein and Fat Concentrations in Cheese Milk.

Lot	% Protein	% Casein	% Fat			C/F Ratios		
			1	2	3	1	2	3
A	3.33	2.54	3.62	3.85	4.00	0.702	0.668	0.640
B	3.48	2.71	3.89	4.02	4.20	0.699	0.669	0.645
C	3.87	3.02	4.22	4.48	4.68	0.711	0.679	0.647

Concentration of milk casein formed  $77.70 \pm 0.44\%$  of milk protein. This falls within the range of 71.4 to 87.1% reported by Cerbulis and Farrell (1975) for casein fractions in milk.

plate attached to a vertical rod. The plate was immersed in milk and oscillated vertically during milk clotting. The force required to move the plate was recorded as a direct measure of curd firmness. The instrument was removed just before the curd was cut.

Sampling: Milk samples were taken from the cheese vat just before starter culture was added. All whey collected at draw was weighed, mixed thoroughly and sampled. Whey drippings during pressing of curd was collected, weighed and sampled. Cheese samples were taken from corners and center of cheese blocks immediately after removal from press. Whey and milk samples were frozen and cheese samples stored at 32F till analyzed.

Compositional Analysis: Raw milk from each lot was tested for fat and protein with a Multispec IR Analyzer (Berwin Co., England). Results of this analysis were used in standardizing the milk using a value of 0.78 x protein for casein. Samples obtained from the cheese vat were also analyzed on the IR. However, actual compositional analysis reported was obtained by determining milk fat with the Babcock procedure and cheese fat by modified Babcock procedure (Van Slyke and Price, 1952) using 10 ml boiling water instead of 9 ml of hot water. Whey fat was determined by the Mojonnier modification of the Roesse-Gottlieb method (Fay, 1959). Milk protein was determined by analyzing milk samples for total nitrogen. Non protein nitrogen and non casein nitrogen were obtained by procedures of Rowland (1938) and Cerbulis and Farrell (1975) with some modification where filtration was done with a Watman 42 filter paper followed by Gelman 0.45 millipore filterpaper to ensure that filtrate was totally devoid of casein. Casein nitrogen was calculated as the difference between total nitrogen and non casein nitrogen. Cheese

This study was designed to investigate the effect of C/F ratio on fat recovery in Cheddar cheese and show the limits of C/F ratio within which the Van Slyke formula for predicting cheese yields will hold. Effects of milk composition on curd firmness at cutting, cheese composition and resulting cheese yield were also determined.

#### MATERIALS AND METHODS

Cheese making: Traditional Cheddar cheese was made from three experimental lots (A, B, and C) of milk at U.S.U. Dairy Products Laboratory between January and April, 1984. Lot A was Holstein milk, lot B was Jersey and lot C was a 50:50 blend of A and B. Each lot was pasteurized (145F, 30 min), cooled, and part of it separated with a 514 Delaval Separator. Separation was at 100F to avoid churning of fat in cream. Pasteurized milk was divided into 3 batches and standardized to the desired C/F ratio by addition of separated cream or skim milk (Price and Calbert, 1951; Price, 1953) then stored at 36F till used the following day.

Four hundred and ten (410) pounds of each standardized milk was accurately weighed into 650 lb. cheese vats and cheese was made by the normal 4.5h method using 0.7 - 0.8% freshly prepared whey-based, pH control lactic starter culture containing a mixture of 2 strains (UC310 and UC77) of Streptococcus cremoris. Cultures were obtained from the Department of Nutrition and Food Sciences culture bank. Acid development was monitored in all lots to ensure a uniform pH of 5.4 at milling. Milled curds were hooped into 20 lb cheese hoops and pressed in a horizontal hydraulic press overnight at 50 psig (344.7 kPa).

Curd firmness at cutting was measured with a Vatimer. This is a device developed at U.S.U. and consists of a horizontal circular

## INTRODUCTION

The value of milk to a processing plant is determined by the efficiency of recovery of milk constituents in cheese making. Knowledge of milk constituent recovery is important in determining quality, yield and price of cheese milk. Intense competition among cheese manufacturing companies has necessitated the need to know both theoretical yields and actual yields in order evaluate plant performance satisfactorily.

A number of formulas have been suggested for estimating cheese yield (Davis, 1965). However, they differ in assumptions made regarding losses of fat and casein in whey and final moisture content of cheese. The Van Slyke formula (Van Slyke and Price, 1952) is most commonly used in the U.S. to predict cheese yields. Variations in milk composition across country has led to questions about the accuracy of the formula, as used in different parts of the country.

U.S. standards for Cheddar cheese require at least 50% fat in the dry matter (FDM) and at most 39% moisture. There is no legal limit to the maximum percent FDM that cheese can have. However, there is a practical limit above which the moisture content must be reduced and other cheese properties changed. Hence, casein to fat (C/F) ratio is important in controlling quality and yield of cheese (Chapman, 1981; Lundstedt, 1979; Phelan, 1981; Price and Germain, 1931).

Good cheese is made at high fat levels but concentration of casein must be increased to balance amount of fat present. Hillers, et al. (1980) have reported an economic advantage in using milk high in protein for cheese making. They also reported that the value of additional fat in milk used to manufacture hard cheese is greater than its value in butter.



Casein/Fat Ratio and Fat Recovery in Cheddar Cheese

by

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was decided to manipulate the extent of HT by changing the holding time, rather than the temperature, as this was easier to control. Extreme care must be taken to insure consistent acidification of the skim milk prior to UF. Slight deviations in pH resulted in significant variances in the degree of HT and the curd forming ability of the retentate.

Cottage cheese manufactured from PA, HT 16% TS retentate cut well in the vat, was not overly fragile or tough, and cooked normally to a final temperature of around 125 F. It was lower in TS than curd made from non-PA retentate, and absorbed dressing well, but not to the extremes mentioned earlier. A low solids dressing was designed to dress the UF curd, containing 16% TS and 12.5% butterfat. Retentate cottage cheese dressed at 60:40 (curd to dressing) met all minimum legal standards.

the curd matrix.

Total calcium in 16% TS retentate was reduced by ultrafiltering pre-acidified (PA) skim milk at pH 5.8. Curd made from PA, HT retentate did not elevate its pH with storage, or exhibit any of the translucency defects previously described. Table I shows that analysis of curd samples for calcium by atomic absorption methods revealed near normal levels of calcium in the PA 16% TS retentate curd, while 16% TS HT retentate curd samples contained 4-6x the amount of calcium on a dry weight basis, depending on the cutting pH.

Table 1. Effect of heat-treatment and pre-acidification on the calcium level of 16% TS retentate curd.

Curd type	pH @ cutting	%Ca D.W.B.
heat-treated	4.90	0.616
heat-treated	4.95	0.730
heat-treated	5.03	0.930
pre-acidified	4.84	0.164
conventional skim milk*		0.158

\*USDA Handbook 8-1, p.14

Sixteen percent TS retentate ultrafiltered from PA skim milk at pH 5.80 and 120 F formed a softer curd than non-PA retentate, but still it did not cut easily with 1/4" harps. A less severe HT was worked out to modify the curd strength suitable for conventional cutting. It

mealiness. It did not appear that rennet improved curd stability.

Of all the treatments experimented with, alteration of pH at cutting had the greatest effect on curd quality and stability. It was found that translucency did not develop when the curd was cut at pH 4.8, instead of pH 5.0, and cooked to a higher temperature. However, these adjustments produced an inferior curd which was mealy in texture. Lowering the pH at cutting eliminated translucency, but created mealiness; clearly, this was no solution.

Two major questions were raised in the course of trying to find solutions. First, why would HT retentate curd turn translucent, while non-HT curd exhibited no such defect? Second, what caused the pH in the curd to elevate, even with acidified dressing at a lower pH than the curd?

It appeared that the curd was being buffered up to a higher pH with storage. Brule (1974) reported that during UF the amount of colloidal calcium phosphate increased proportionately to the amount of protein in the retentate. Brule and Fauquant (1981) reported that UF did not alter the saturation equilibrium established between the aqueous and colloidal phases of milk. However, it is known that HT of milk causes reduction of both the total soluble and ionic calcium, which is partially attributable to a conversion to the colloidal state (Parry, 1974). Therefore, it was concluded that main differences between HT and non-HT retentate curd must be the concentration of colloidal calcium phosphate, and the presence of heat-denatured whey protein in

solution, accounting for the visual and textural problems. First, acidified rinses and dressings were tried. Emmons (1959) reported H<sub>1</sub> denatured the whey proteins, causing the AC endpoint to be raised to a higher pH. This in turn produced a curd with a higher pH, which was corrected for by adding acidified dressing. This solution proved ineffective with HT retentate curd, regardless of the acidification level of the dressing or the ratio of applied dressing to curd. Dressings were acidified to pH 5.5, 5.3, 5.0, and 4.8; application rates of dressing to curd were tried at 40:60, 50:50, and 60:40. Acidified rinses and dressing did help to lower the pH of the finished cottage cheese, but did not halt the shift in pH or the curd's dissolution.

Cottage cheese curd made from 16% TS appeared to cook out quickly in the vat, became quite firm, and was typically high in TS. In an effort to keep TS close to legal minimum standards (20% TS for dry curd), retentate curd was cooked to a final temperature of 110-115 F. After experiencing translucent curd, it was decided to raise the final cook temperature back to the traditional 125-130 F to test if the higher cook would cause the curd to be more stable. Final cooking temperature affected the TS and texture, but with high pH curd it did not inhibit the translucency defect.

The effect of rennet on curd formation, cuttability, and curd stability was investigated. Full strength rennet was added at a rate of 0.025ml/#TS and did not adversely affect cuttability, but did cause tighter shrinking of the curd during the cook, higher TS, and

## PROGRESS REPORT:

When this research project was initiated, the principle difficulty in producing a DA curd from high solids retentate was cutting the set coagulum. Non-modified retentate curd was extremely tough and rubbery. It was found that a proper heat-treatment (HT) of the retentate reduced the retentate's curd forming ability, and allowed acceptable cutting with conventional harps.

Last year it was reported that a pilot process for manufacturing high quality cottage cheese from 16% TS retentate had been developed. Unfortunately that announcement was premature, as it was later observed that a repeatable defect in the UF cottage cheese occurred with storage. Cottage cheese curds made from HT retentate cut and cooked well, appeared normal in every respect, and exhibited good body and texture. However, over time a gradual translucency developed in the curd. Several other problems accompanied the apparent loss of opacity. First, the pH of the curd rose from the cutting pH of about 5.0 to 5.2 or 5.3. Second, the texture of the cottage cheese deteriorated from a good meaty texture to a soft, pasty texture. Third, UF curd absorbed all available dressing, causing the final product to appear dry and sticky.

Several manipulations of the process were experimented with to try and stop the development of the translucency defect. It was postulated that the pH of the curd was causing the casein to go back into

# PRODUCTION OF COTTAGE CHEESE USING ULTRAFILTRATION TECHNIQUES

by Ron Raynes

## INTRODUCTION:

Interest in new technology applied to traditional dairy products has motivated research in ultrafiltration (UF). Cottage cheese is a fresh, unripened cheese manufactured from skim milk using either starter fermentation or direct acidification (DA). Application of UF in the production of cottage cheese has been experimented with at USU to determine whether a high quality curd can be made with conventional equipment. Earlier research at USU by Narasimhan indicated a possible yield increase of 12% using 16% total solids (TS) retentate. This is thought to be due to entrapment of whey proteins in the curd and reduced syneresis of whey. Other benefits from the use of UF in the manufacture of cottage cheese include increased vat efficiency, and potential application in continuous processes. An increasing trend towards on-farm UF to reduce trucking and tax expenses also points to the need for understanding membrane technology, and the physicochemical parameters associated with retentate products. Current research at USU has involved perfecting a pilot process for comparing yield differences between traditional and UF procedures.

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curd firmness offers the potential for reducing losses of cheese yield. Results of this study demonstrated variations in curd firmness at cutting with milkfat. At constant protein levels, increased milkfat (low casein/fat ratio), resulted in a more rigid curd (Table 5).

Table 5. Coagula firmness of cheese milk

Protein	Casein/Fat Ratio	Curd Firmness
3.33	0.640	1.895
	0.668	1.225
	0.702	0.730
3.48	0.645	1.024
	0.669	1.006
	0.699	0.840
3.87	0.647	1.772
	0.679	1.412
	0.711	1.378

<sup>a</sup>=Adjusted for uniform moisture of 37%

It is difficult to relate curd firmness at cutting to cheese yield because coagula were not cut at the same firmness but 30 minutes after renneting. Curd firmness at cutting correlated with cheesefat ( $r = 0.8$   $p < .01$ ).

#### CONCLUSIONS

Efficiency of recovery of milkfat and casein, as well as concentration of fat and casein in cheese milk largely determine cheese yield.

In Cheddar cheese manufacture, there is no significant effect of casein/fat ratio on fat recovery within casein/fat ratios of 0.64 to 0.71. The original Van Slyke equation does a good job in estimating the magnitude and direction of change in cheese yield with variations in casein/fat ratios. Within the limits of casein/fat ratio of 0.64 to 0.71 the modified Van Slyke equation predicts cheese yield quite accurately.

Curd firmness at cutting is related to the amount of fat in cheese milk at all protein concentrations. The higher the fat content, the firmer the curd.

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Running Head: Proteinase Negative Cultures

Differentiation of Proteinase Positive and Negative Lactic Cultures  
Using Growth in Skim Milk

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Key Words: Proteinase Negative  
Lactic  
Streptococci  
Assay

Footnotes:

1. Contribution Number 2933 of the Utah Agricultural Experiment Station. Approved by the Director.
2. Mention of companies or products does not constitute endorsement by Utah State University over similar products not mentioned.

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# ABSTRACT

A sterile, nonfat, dry-milk substrate with beta glycerophosphate was used to differentiate proteinase-positive and -negative lactic streptococci. Fifteen pairs of both cell types were incubated in this medium 24 h at 22°C. Proteinase-positive cells produced from six to 27 times the cell mass of the proteinase negative variants. Streptococcus cremoris proteinase negative isolates produced a significantly larger cell mass than did S. lactis isolates. Cell mass production from proteinase-negative variants did not correlate with high-cell-mass producing parent strains. When mixtures of the two cell types were grown in the medium, from 10 to 50% of proteinase-positive cells were required for growth and acid production to equal that of 100% proteinase-positive cells. It was possible to detect 2% proteinase positive cells in a proteinase negative culture in all strains examined.

## INTRODUCTION

With the suggestion made for exclusive use of proteinase-negative (Prt-) lactic streptococcal variants in cheese making (10), a method is needed to differentiate them from their proteinase-positive (Prt+) counterparts (4,12) and to detect when Prt+ cells are present in Prt- cultures. Various media and methods of differentiating Prt- and Prt+ isolates exist but are time consuming and are not reliable with some strains (1,2,3,4,7). A simple, and reliable method of differentiating between Prt- and Prt+ isolates was investigated using a buffered substrate that contained only the nitrogenous matter available in sterilized nonfat dry milk (NDM).

## METHODS AND PROCEDURE

### CULTURES

The Prt+ and Prt- cultures of Streptococcus lactis and S. cremoris were from the frozen stock maintained at the Department of Nutrition and Food Sciences at U.S.U. The Prt- variants had been previously isolated on glycerophosphate-milk agar (8) and by testing the isolates' ability to coagulate sterile 10% NDM within 24 h (10).

The Prt+ cultures were propagated in sterile, 10% NDM. Prt- cultures were propagated in NDM fortified with .5% yeast extract (YNDM). The media were sterilized at 121°C for 15 min. Frozen cultures were stored at -40°C and working cultures were transferred weekly by inoculating .1 mL of coagulated culture into 10 mL of the appropriate media and storing at 4°C. They were incubated 24 h at 22°C prior to use or transfer.

# 1 BUFFERED MILK SUBSTRATE

2 A stock solution containing 95 g of Grade II sodium beta glycerol  
3 phosphate (Sigma Chemical Co<sup>2</sup>, St. Louis, MO) and 100 mL deionized water  
4 was mixed and dispersed in 20-mL quantities into 100-mL dilution bottles  
5 and autoclaved (11). Buffered NDM substrate (BNDM) was prepared by  
6 adding .6 mL of the buffer stock solution to 10.0 mL of NDM (pH = 7.2).

# 8 CELL MASS DETERMINATIONS

9 Cell mass determinations were made turbidometrically, using the  
10 modified procedure (10) of Kanasaki et al. (7).

# 12 INOCULATIONS

13 Refrigerated cultures were incubated for 24 h at 22°C before  
14 inoculations were made. The Prt- cultures were inoculated by adding .1  
15 mL of coagulated culture to 10.6 mL of BNDM. Volumes of Prt+ cultures  
16 inoculated into BNDM were based on the cell mass of a 1% inoculum of the  
17 corresponding coagulated Prt- culture so that the initial cell numbers  
18 of both Prt+ and Prt- cultures of a given strain would be approximately  
19 equal. Since the cell mass attained by the Prt- cultures was usually  
20 larger in YNDM, the inoculum amounts of coagulated Prt+ cultures were  
21 raised accordingly. Usually .12 to .17 mL were equivalent to the .1 mL  
22 of Prt- inocula.

# 24 MIXED PRT+ AND PRT- CULTURES

25 Mixtures of Prt+ and Prt- cultures were made by determining the  
26 initial cell mass of each and adjusting inoculations such that different  
27 proportions of Prt+ and Prt- cells existed in 1% inoculum in BNDM.



1 Mixtures containing varying percentages of Prt+ cells (0-100%) were  
2 tested in triplicate. Asymptotic curves were calculated to estimate the  
3 proportion of Prt+ cells equivalent to 100% Prt+ cells in cell mass and  
4 acid production, and also to estimate the percentage of Prt+ cells in a  
5 Prt- culture.

## 6 RESULTS AND DISCUSSION

### 7 PRT- DIFFERENTIATION

8 The cell mass attained by the Prt+ cultures in BNDM was from six to  
9 27 times as great as that attained by the corresponding Prt- cultures  
10 (Figure 1). The mean (x) and standard deviation(s) for cell mass were  
11  $6.41 \pm 1.83$  for Prt+ and  $.44 \pm .15$  for the Prt- variants. They were  
12 significantly different at P .001. The values the four S. lactis Prt-  
13 variants were  $.30 \pm .05$  and the ten S. cremoris Prt- variants were  $.49 \pm$   
14  $.13$ . The procedure allowed the differentiation of S. lactis from S.  
15 cremoris Prt- strains at P .01.

16 The overall correlation coefficient between the two types of cells  
17 was  $R=.00$ , thus the proportion of proteinase activity after isolation of  
18 the Prt- variant did not relate to the proteinase activity of the parent  
19 cells. The slow growth of the Prt- variants is due to the low  
20 concentration of free amino acids and peptides in milk and the  
21 unavailability of these by-products (normally released by Prt+ cells) in  
22 lactic cultures. Cell mass differences were accentuated by the addition  
23 of sodium beta glycerophosphate buffer, which reduced growth inhibition  
24 of the Prt+ cultures. The proteinase activity differences among Prt+  
25 strains were much more evident than were the differences among Prt-  
26 cells. Proteinase assays such as recently proposed by Church et al.

1 (1) may prove helpful in efforts to better quantitate the activity of  
2 Prt- variants.

3

#### 4 MIXED PRT+ AND PRT- CULTURES

5 About one to two percent of the cells in single strains of lactic  
6 streptococci gradually lose their plasmid-linked proteinase activity  
7 (4,6). Thus, frequent transfer of a culture and attempts to carry  
8 single strains over long periods of time will produce cultures with  
9 variable mixtures of Prt+ and Prt- cell types. Since the Prt+ cells  
10 produce an excess of nitrogenous matter that can be utilized by Prt-  
11 cells, no change in acid production rates in a culture are detectable.  
12 When the proportion of Prt+ cells drops to a level where they are unable  
13 to sustain the needs of the increased proportion of Prt- cells, however,  
14 the acid production rate is significantly reduced. This level has been  
15 considered to be 10 to 20% of Prt+ cells (4,12).

16 Tests of known mixtures of Prt+ and Prt- cultures of six strains  
17 showed that a range of from 10 to 50% of a culture must be Prt+ for  
18 growth and acid production to equal that of a Prt+ culture (Figure 2).  
19 Some of the strains required higher Prt+ concentrations to restore full  
20 Prt+-like activity than had been previously reported (4,12). This is  
21 indicative of the wide variance in ratios of Prt+/Prt- cells that might  
22 exist before measureable losses of culture activity occur. It also  
23 suggests that one Prt+ cell can produce sufficient available nitrogen to  
24 support the growth of from one to nine Prt- cells, depending upon the  
25 strain.

26 For a given strain it should be possible to estimate the proportion  
27 of Prt+ cells in a blend below about 50%. This test appears to be

1 simple and effective even though it represents an indirect measure of  
2 proteolytic activity.

3 Wright et al. (13) successfully used this type of test to predict  
4 inoculum levels required to produce desired changes in pH during cheese  
5 manufacture. Such a technique should find application in cheese culture  
6 selection. Additionally, those strains producing the largest cell mass  
7 may be desired where accelerated ripening is desired (5). Conversely,  
8 those with lowest growth potential would be useable in stored cheese  
9 where ripening is discouraged, or in cottage cheese where maximum yield  
10 is desired (9).

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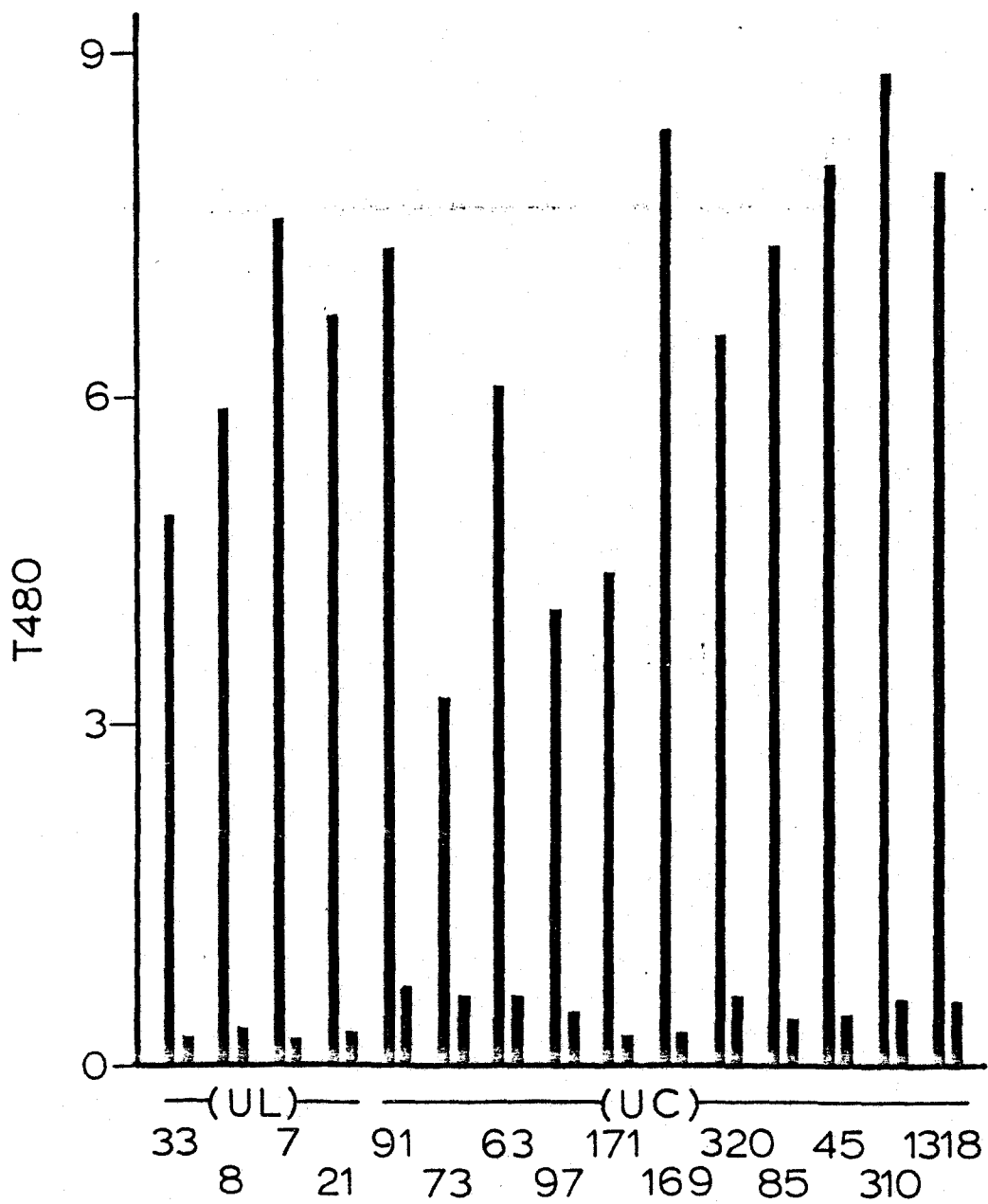
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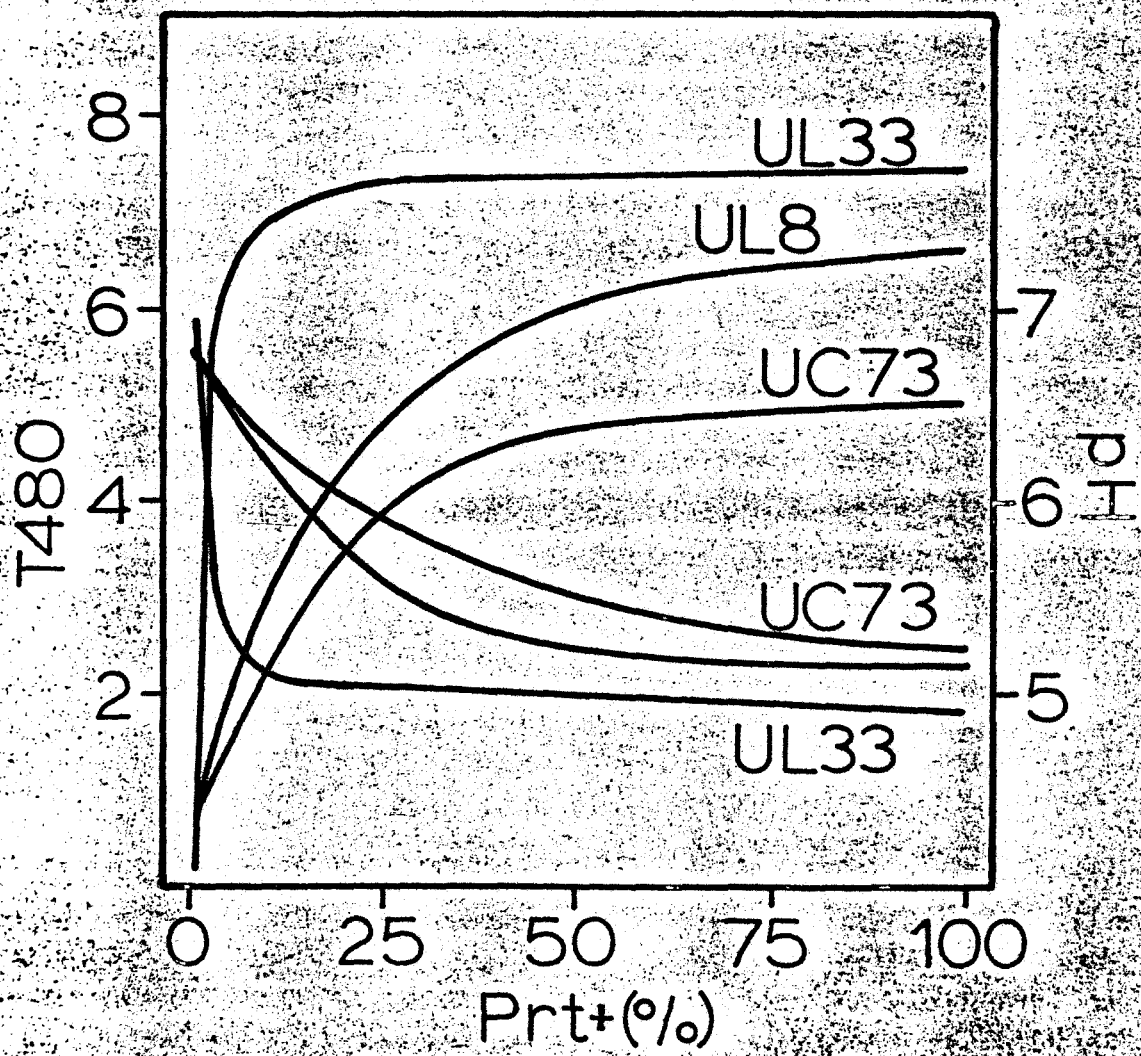
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## FIGURE TITLES

Figure 1. Cell mass (T480) obtained by Prt- and Prt+ isolates grown in buffered NDM substrate (24 h at 22 C).

Figure 2. Effect of different ratios of Prt- to Prt+ cultures on the final cell mass and pH attained after a 24-h incubation at 22 C in buffered NDM substrate. Of the six strains tested, strain UL33 required the fewest Prt+ cells to demonstrate 100% Prt+ activity while strain UC73 required the most. Other strains had similar curves and fell between these two.







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Running Head: Protease Negative Cultures

Paired and Single Strain Protease Negative Lactic  
Streptococci for Cheese Manufacture

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## ABSTRACT

After propagation in pH controlled media, pairs of protease negative lactic streptococci produced acid better than component strains. Some protease positive pairs were balanced with 1:1 ratios and others produced 9:1 ratios after one propagation.

Acid production by protease negative variants was unaffected by homologous phage (i.e. remained at 100% of uninoculated controls) either when paired or in single strain culture if the total numbers were about four times that of protease positive cells. Single strain cultures of protease positive cells lost significant activity with added bacteriophage. As bacteriophage concentration increased from  $10^1$  to  $10^9$  per milliliter the activity dropped to 72 to 1% of the controls. When paired, the rate of acid production improved and was 90 to 62% that of protease negative cells as bacteriophage increased from  $10^1$  to  $10^9$ .

Antibiotics affected acid production of both cell types but the protease positive were more inhibited. Pairing did not improve the performance of either culture with regard to added antibiotics.

## INTRODUCTION

Paired strains of lactic cultures are used effectively in the manufacture of Cheddar cheese (7,14). Most plants in New Zealand and over forty plants in the United States now use only one pair of lactic streptococci (Robert Lawrence and Reed Ernstrom, Personal Communications, 1983). Single strain cultures have been used in Australia (6). Fewer culture control problems exist when the number of strains is minimal (1,6,7,14,17). Suggestions that protease negative ( $\text{Prt}^-$ ) lactic cultures be used exclusively (13) prompts reconsideration of the need for bacteriophage (phage) control through the use of pairs or culture rotation. This paper compares the performance of paired and single strain cultures when challenged by phage or antibiotics.

## MATERIALS AND METHODS

### Cultures

Both  $\text{Prt}^+$  and  $\text{Prt}^-$  cells were isolated from stock cultures on milk agar medium (13). The  $\text{Prt}^+$  isolates were propagated in sterile reconstituted nonfat dry milk (RNDM). A 10% solution was prepared, dispensed into 15 x 150 mm test tubes, capped and sterilized at 121°C for 15 min. The  $\text{Prt}^-$  isolates were propagated in the same medium fortified with .5% yeast extract. All strains were subcultured weekly by inoculating 1% of a freshly coagulated culture into sterile substrate, storing at 5°C, and incubating at 22°C for 18 to 20 h just prior to use.

### Culture Activity Test

Protease negative isolates were propagated singly and in pairs in whey-based medium under pH control (12). The cultures were inoculated at .5, 1.5, 2, 5 and 10% into sterile RNDM substrates and incubated in a

1 Pearce activity test (10). Change in pH was determined. Percent  
2 inocula required to obtain a pH 1.1 change was calculated using the  
3 equation  $Y = aX^b$  where  $Y = \% \text{ inoculum}$  and  $X = 1.1 \text{ change in pH}$ . The  $a$   
4 and  $b$  values were the constants calculated for each strain.

#### 5 Dominance Test

6 Pairs of  $\text{Prt}^+$  cultures were propagated together through one cycle  
7 in an external pH control system (12). Each strain was inoculated at  
8 .5% for 1% total volume and the substrate was controlled at pH 6 to 6.2  
9 for 12 h at 27°C. The culture was diluted serially so as to produce  
10 slightly over 100 colonies per plate and plated on M17 (16) agar.  
11 Twenty colonies were parallel streaked on an M17 plate (5). Phage stock  
12 solution, homologous for one of the paired strains, was streaked  
13 perpendicularly across the culture streaks. After incubation 48 h at  
14 30°C, the lysed and unaffected streaks were counted and the percentage  
15 of each strain in the pair was calculated.

#### 16 Phage Filtrates

17 Homologous phage isolates obtained from cheese plants during  
18 evaluation of a defined culture program (14) were prepared in stock  
19 solutions containing from  $10^8$  to  $10^{10}$  plaque forming units per  
20 milliliter using the technique of Heap et al. (5).

#### 21 Phage Test

22 Bromcresol purple was added to RNDM at .015% before sterilization  
23 and designated BNDM. Each well in one row of a Microtiter<sup>3</sup> (Cooke

24  
25 <sup>3</sup>Mention of products or companies does not represent endorsement by  
26 Utah State University over products not mentioned.

1 Engineering Inc. Alexandria, VA) plate was filled with .225 mL BNDM  
2 previously inoculated with either 2%  $\text{Prt}^+$  or 8%  $\text{Prt}^-$  cultures (13). A  
3 .025 ml Microtiter Diluter was filled with phage stock solution and  
4 mixed into the first well in a row. The phage stock was diluted  
5 decimally to  $10^{-9}$  and mixed within the row of inoculated BNDM wells  
6 using the Diluter. The last well was an uninoculated control. All  
7 wells were sealed with cellophane tape and the plates were immersed in a  
8 water bath controlled at Cheddar cook temperatures (10). Color changes  
9 in the wells were compared after 5 h as indicative of phage or  
10 antibiotic inhibition. A 4 mm diameter combination pH electrode  
11 (Sensorex, Westminster, CA) was used for pH measurements in the wells.  
12 The change in pH of the inoculated substrates was divided by the pH  
13 change in the phage- or antibiotic-free controls and multiplied by 100  
14 to determine percent activity.

#### 15 Phage Series Test

16 Three  $\text{Prt}^+$  and three  $\text{Prt}^-$  variants, isolated from common parent  
17 strains, were subjected to homologous phage stock in a seven culture  
18 series (4). A .1 mL phage stock and .1 mL homologous phage stock were  
19 added to sterile 10 mL RNDM inoculated with .2 mL culture. The  
20 inoculated substrates were incubated through a Pearce (10) test  
21 and the changes in pH were compared between substrates with and without  
22 added phage.

#### 23 Antibiotic Test

24 Stock solutions of penicillin G sodium salt, erythromycin and  
25 dihydrostreptomycin (Sigma Chemical Co. St. Louis, MO) of .01M were  
26 prepared in phosphate buffer (2,9). These solutions were used like the  
27 phage solutions for the antibiotic studies.

## RESULTS AND DISCUSSION

### Culture Activity Test

When  $\text{Prt}^-$  strains were paired prior to propagation in whey-based medium under pH control the acid production activity in RNDM was better in all but one example than when single strains were used (Figure 1). It was not determined whether dominance of the active strain occurred or if both grew in a balanced condition. Pairs C63/C73 and C63/L33 performed better than either strain alone suggesting synergism. Conversely, pairs C161/C63 and C320/C73 showed such discrepancy between single component strains that synergism was not suggested. Pair C63/C73 response suggests protease complementation as if in a cis-trans position. Such prompts additional study.

### Dominance Test

A pair of  $\text{Prt}^+$  strains, C7/C2B, retained 45:48 and 49:51% cell balance after one (50:50) propagation in pH controlled bulk culture. Conversely, pairs of C2A:134 and BA1:134 produced final ratios of 83:17 and 90:10 respectively. When the former was inoculated in 1:20 ratio to reduce dominance the ratio was still 66:34.

Pairs which remain balanced during bulk preparation should be sought. An assay involving a Spiral Plater appears to be a simple and valuable method to identify compatible pairs (15).

### Phage Series Test

Mean activities for three single strain  $\text{Prt}^+$  lactics (UL8, UL21 and UC171) were 69 and 32% of phage-free controls in the first two cycles of the phage series test (4). The mean values for  $\text{Prt}^-$  variants of the same strains were 84, 86, 86, 92, 98, 97 and 94% through seven cycles.

1 The  $\text{Prt}^-$  cells, in sufficient numbers to produce ample acid, appear  
2 indifferent to phage and improve in activity as phage is diluted out  
3 from one cycle to the next.

#### 4 Phage Test

5 The lower bars in Figure 2 are mean values of percent activities of  
6 single and paired lactic strains (UL7, UL8, UL21, UL33, UC91 and UC171)  
7 when challenged with one homologous phage. The stacked bars represent  
8 one standard deviation above the mean. The  $\text{Prt}^-$  cultures experienced no  
9 reduction of activity at any level of homologous phage inoculation  
10 though the strains were sensitive to the phage used. Both single and  
11 paired strains retained activity indicating no advantage to blending.  
12 Cell crowding, resting cells with very low growth rates (13), and the  
13 need for acid production to meet energy requirements probably account  
14 for the ability to produce acid and remain indifferent to disturbing  
15 phage.

16 Single strains of  $\text{Prt}^+$  cells were severely reduced in ability to  
17 produce acid as phage concentration was increased (Figure 2). The  
18 coefficients of variation were very large (16, 34, 48, 43, and 100%  
19 respectively) indicating the wide range of effects when one strain is  
20 infected and the relative infectivity of phage isolates. When pairs of  
21 the  $\text{Prt}^+$  cells were infected with one phage race the activity improved  
22 markedly. The coefficients of variation were also significantly  
23 reduced (7, 10, 11, 15, and 19% respectively). The improvement of using  
24  $\text{Prt}^+$  pairs over single strains was highly significant ( $p=.01$ ).

25 These data confirm that paired  $\text{Prt}^-$  strains have no advantage over  
26 single strains if cultures can be protected from phage during bulk

1 culture production (11). Both  $\text{Prt}^+$  and  $\text{Prt}^-$  cells would be adversely  
2 affected if phage were inoculated during growth in bulk culture.

### 3 Antibiotic Test

4 The effects of the antibiotics upon single and paired strains are  
5 summarized in Table 1. The differences among antibiotics were highly  
6 significant (2). Both cell types were adversely affected but acid  
7 production by the  $\text{Prt}^+$  cells was more inhibited. Pairing of either  $\text{Prt}^+$   
8 or  $\text{Prt}^-$  cells produced no significant advantage over single strains.

### 9 Conclusions

10 Pairing of  $\text{Prt}^-$  strains does not appear valuable unless it can be  
11 established that synergism is involved (Figure 1) (3,8). The use of an  
12 active single  $\text{Prt}^-$  variant should be adequate to assure normal acid  
13 production in cheese manufacture. The ability to use single  $\text{Prt}^-$   
14 strains (Figure 2) (6) simplifies continuous fermentation since balance  
15 problems associated with paired strains (1, 3, 7), multiple strains (8,  
16 17) or build up of  $\text{Prt}^-$  cells in  $\text{Prt}^+$  cultures (13) are eliminated.

17 Studies are underway to evaluate cheese aging and yield factors  
18 associated with  $\text{Prt}^-$  variants.

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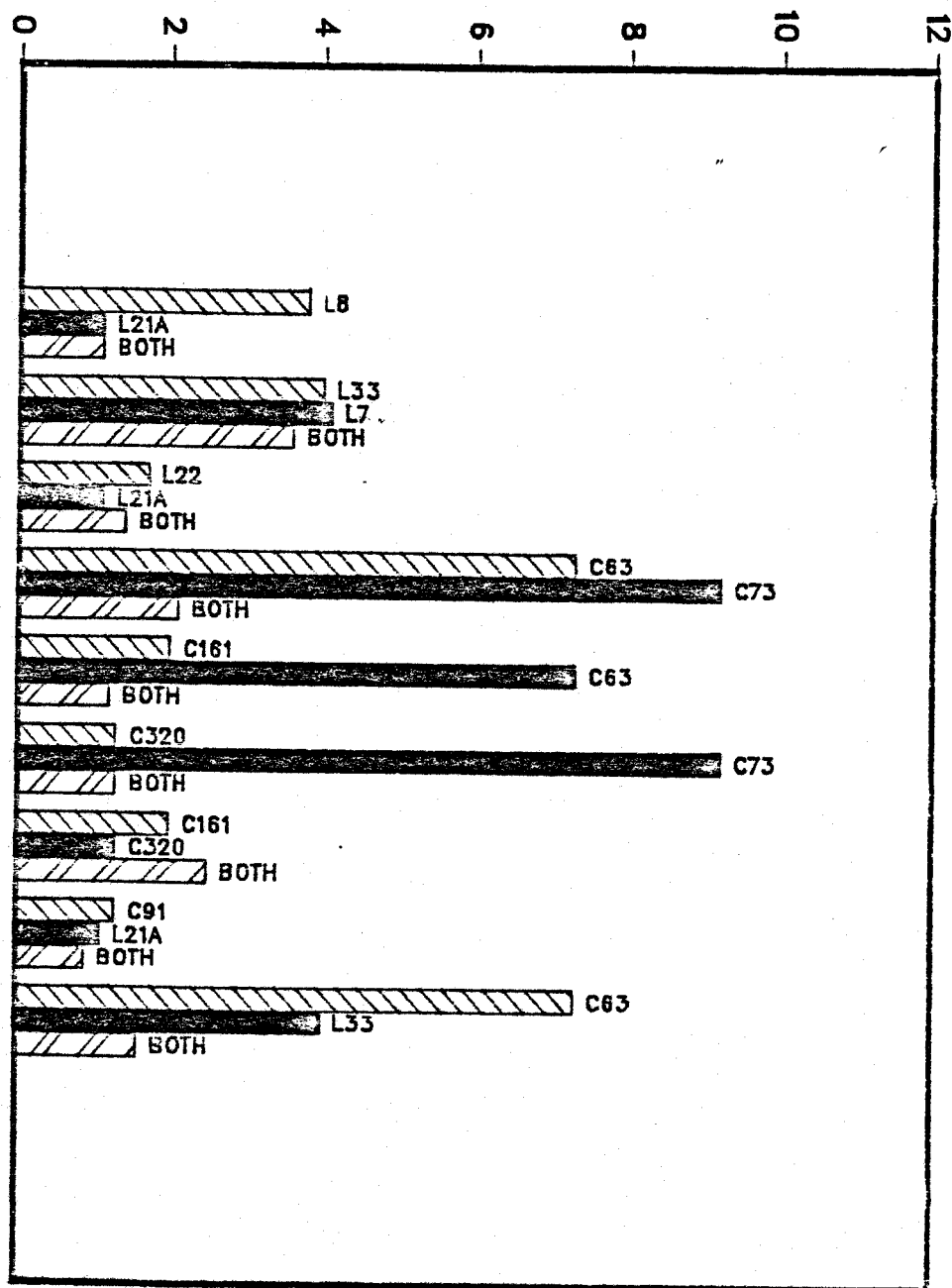
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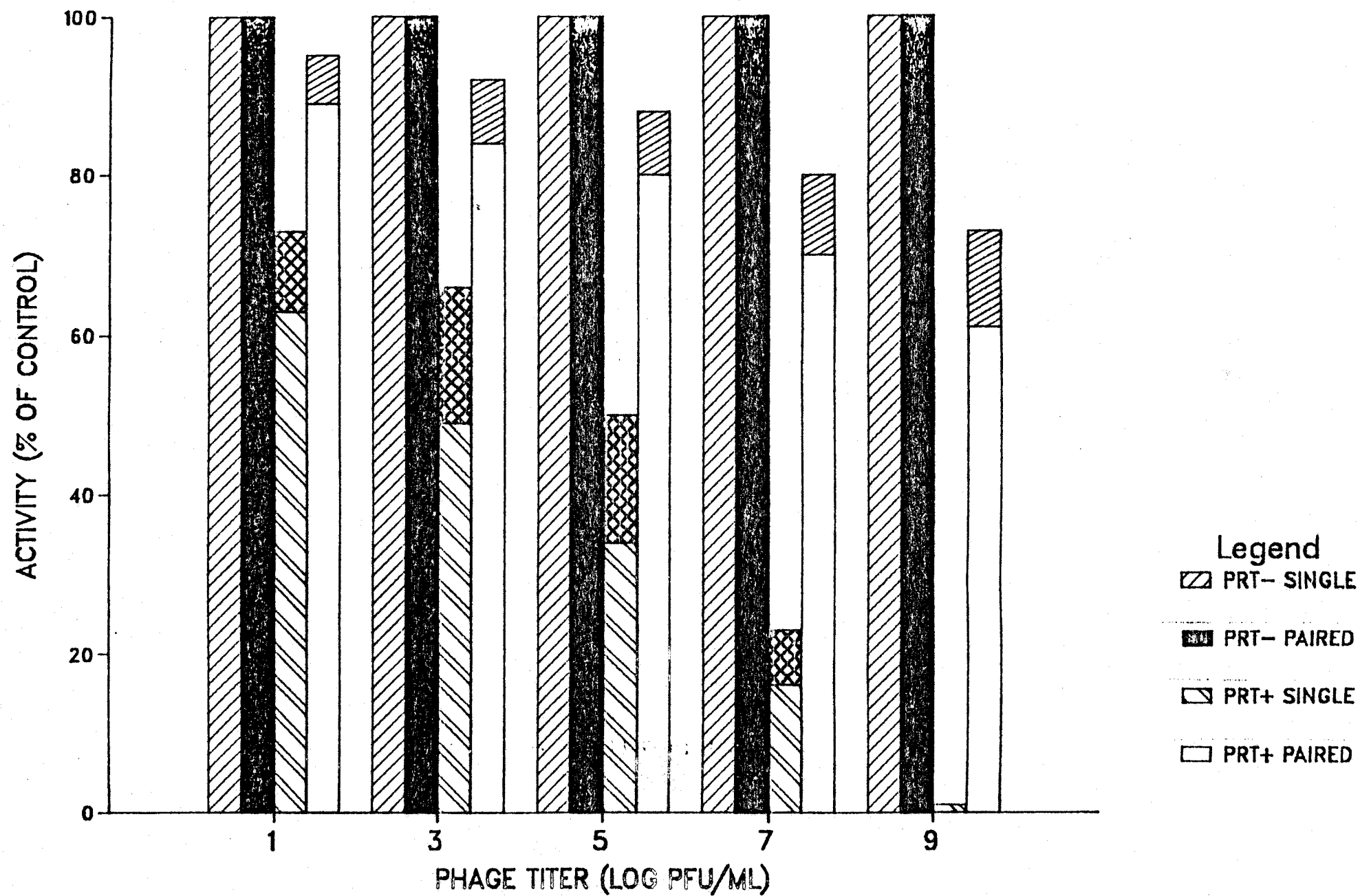
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FIGURE 1. Percent (V/V) of single and paired protease negative lactic cultures required to produce a 1.1 pH change in reconstituted nonfat dry milk medium in the Pearce activity test.

FIGURE 2. Effect of one homologous phage upon percent acid production of single and paired lactic streptococci. Each lower bar represents the mean of 10 evaluations using strains UC73, UC77, UL8 and UL33 and phage stock solutions UP73, UP77, UP8 and UP33. The pairs evaluated included UL33/UL8, UC77/UL8, UC73/UL33, UC73/UL8 and UC77/UL33. The upper, stacked bars represent the positive standard deviations. Activity was measured by evaluating the change in pH in sterile RNDM during the Microtiter activity test.

# PERCENT INOCULUM





# THE MANUFACTURE AND AGING OF CHEDDAR CHEESE MADE WITH PROTEASE NEGATIVE CULTURES

Craig Oberg and Lauris Davis

## Background

A distinction has been made among strains of lactic Streptococci concerning protease activity. Those designated protease positive can coagulate milk at a 1% inoculum level in less than 24 hours when incubated at 22 C. Protease negative or deficient variants take much longer (usually over 48 hours) since they primarily are growth limited by previously available amino acids and peptides for their nitrogen source. Negative cultures have not been used for cheesemaking because of higher vat inoculum levels needed, longer make times, and because more expensive starter is required.

Recent research has established several advantages for the use of protease negative strains. Growth of protease negative strains is severely limited in cheese milk so they are resistant to bacteriophage attack. Kentucky researchers have demonstrated increased yield with some protease negative strains probably since they degrade less casein. Other advantages include better control over acid production in the cheese vat, a reduction in bitter peptides formed during the curing process, and the potential to use higher cooking temperatures to possibly shorten the make time.

## Purpose and Methods

The purpose of this research was to examine the feasibility of making Cheddar cheese from protease negative cultures and to compare its curing to that of Cheddar cheese made from protease positive lactic starter. Cheddar cheese was made with a single protease

negative strain Streptococcus cremoris UC 73- and a frozen commercial protease positive bulk set. The protease positive cheese was used as the control using two different starter media. Nine trial vats containing 640 pounds of milk were made from each starter type using a conventional 4.5 hour Cheddar make procedure. For starter production, seven and one half liter stainless steel tanks were fitted with lids containing an inoculation port, a pH electrode port, and an electric motor driven mixer. The incubation reservoir held four such tanks. The protease positive external pH control medium consisted of 5% whey solids and 1.25% Biolac powder medium. The other protease positive medium contained 11.62% phage inhibitory medium powder. The protease negative medium contained .4% yeast extract, .1% casein hydrolyzate, and 5% whey solids.

The starter media was heat treated with flowing steam in an autoclave. The treatment was comparable to that recommended for use in commercial plants. The incubation temperature was 24 C. One ml of the frozen protease positive concentrate was used for six liters of positive medium and 15 ml of freshly coagulated NFDM was used for 6.5 liters of negative medium. All cultures were ready after 18 hours although the negative cultures could be used after 13-15 hours. In the cheese manufacture activity tests gave estimates for the inocula levels of each starter. The pH controlled whey-base protease positive starter was used at a 0.7% level, the PIM starter at a 1.4% level, and the negative at a 2% level. Earlier work in our lab indicated that an inoculum of 8% pH control culture would be required for protease negative starter culture.

Analyses were run on the milk, cheese, and whey. The milk was tested for fat and protein content with the Multispec infrared milk

analyzer and for total solids with the AOAC method. Milk and cheese fat was also determined using the Babcock fat test. Total nitrogen for each was determined using a Tecator automated kjeldahl machine. Aging analysis on each vat of protease negative and positive cheese was performed for one year to determine any differences between the culture types. Five expert cheese graders judged the cheese at 15, 60, 90, 180, and 365 days of age. Soluble nitrogen as a percent of total nitrogen was determined using the Vakaleris and Price method at 30, 60, 90, 180, and 365 days.

### Results

The cheese manufacture was completed in June of 1983 with the analysis just finished this past month. All the cheese produced had acceptable moisture levels and good body and texture. The make times (time from set to mill) for the protease negative vats had less variability then for the protease positive vats. Since the protease negative cultures were not required to replicate in the cheese vat they were less affected by any extraneous influences.

In the grading results several correlations were observed, the flavor score was closely tied to the total score for the cheese and flavor intensity correlated with the increasing age of the cheese. A comparison of the three treatments versus age for each of the graded characteristics is found in figures 1-6. All of the cheeses graded out well but there are some differences between the treatments as is illustrated. Analysis of variance was run for each grading characteristic and has expected the graders category contained most of the variance for the individual models with several exceptions. Flavor quality as influenced by treatment. Flavor intensity was greatly affected by age, as was curdiness. Duncans multiple range



test (alpha level of 0.05) showed significant differences between the treatments in most categories. The negative cheese was found to have the lowest values for flavor quality and flavor intensity and to have the highest values for body and texture characteristics.

The comparison of the three treatments versus age for soluble nitrogen percent cheese is found in figure 7. The largest correlation was found between soluble nitrogen and age although treatment also played a role in explaining the variability found in the model. According to the Duncans multiple range test there was a considerable difference between the negative cheese and the two positive treatments. A significant difference was also found in the kjeldahl values for the whey. The negative cheese whey contained .955% protein while the two positive cheese wheys averaged .920% protein.

# CHEESE GRADING SHEET

GRADER \_\_\_\_\_

TRIAL \_\_\_\_\_

DATE \_\_\_\_\_

SAMPLE \_\_\_\_\_

ITEM	SCORE	COMMENTS
Flavor		
Body		
Texture		
Total		
Cheese Flavor Intensity		1 2 3 4 5 6 (---> increasing)
Curdiness		1 2 3 4 5 6 (---> increasing)

SAMPLE \_\_\_\_\_

ITEM	SCORE	COMMENTS
Flavor		
Body		
Texture		
Total		
Cheese Flavor Intensity		1 2 3 4 5 6 (---> increasing)
Curdiness		1 2 3 4 5 6 (---> increasing)

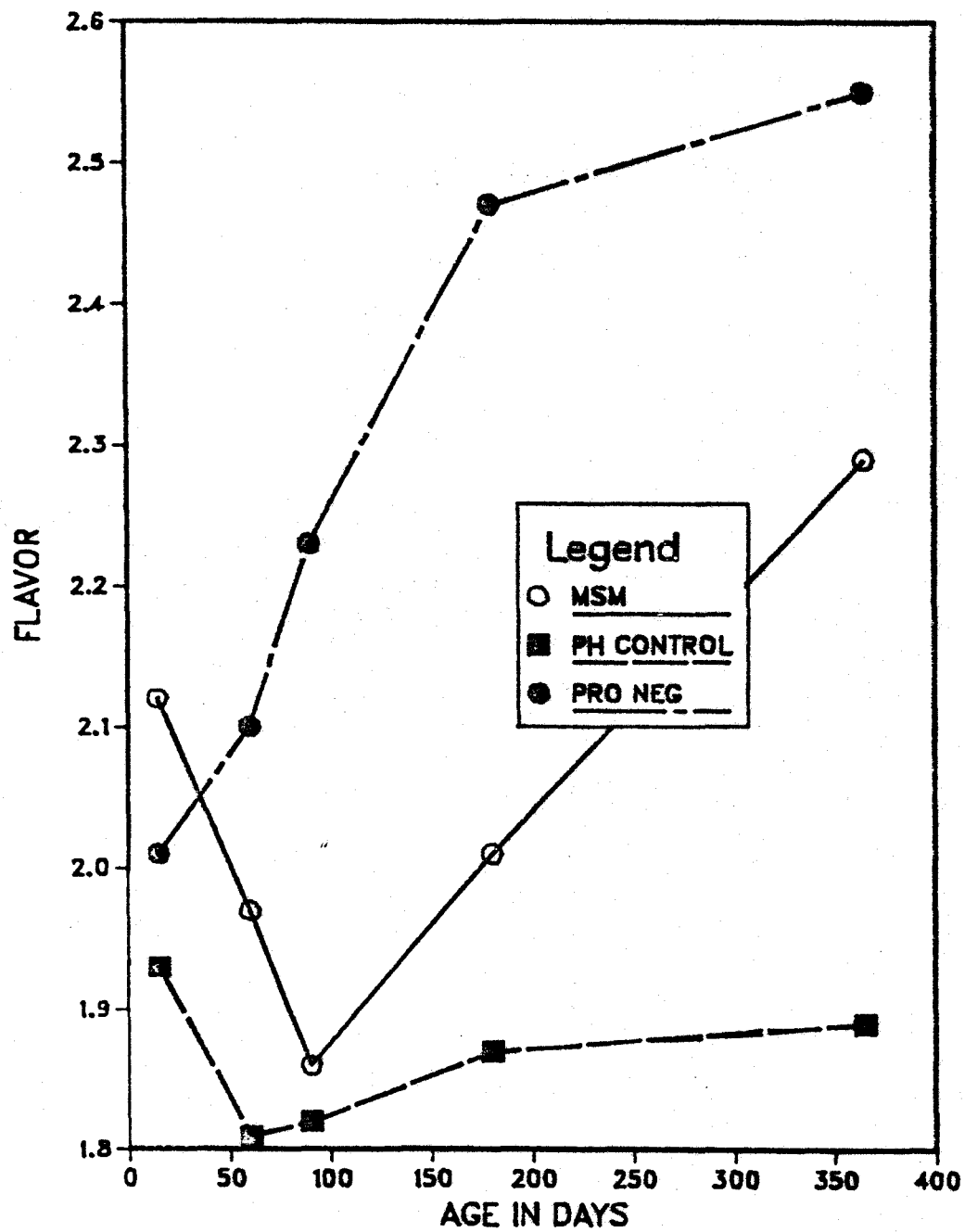
SAMPLE \_\_\_\_\_

ITEM	SCORE	COMMENTS
Flavor		
Body		
Texture		
Total		
Cheese Flavor Intensity		1 2 3 4 5 6 (---> increasing)
Curdiness		1 2 3 4 5 6 (---> increasing)

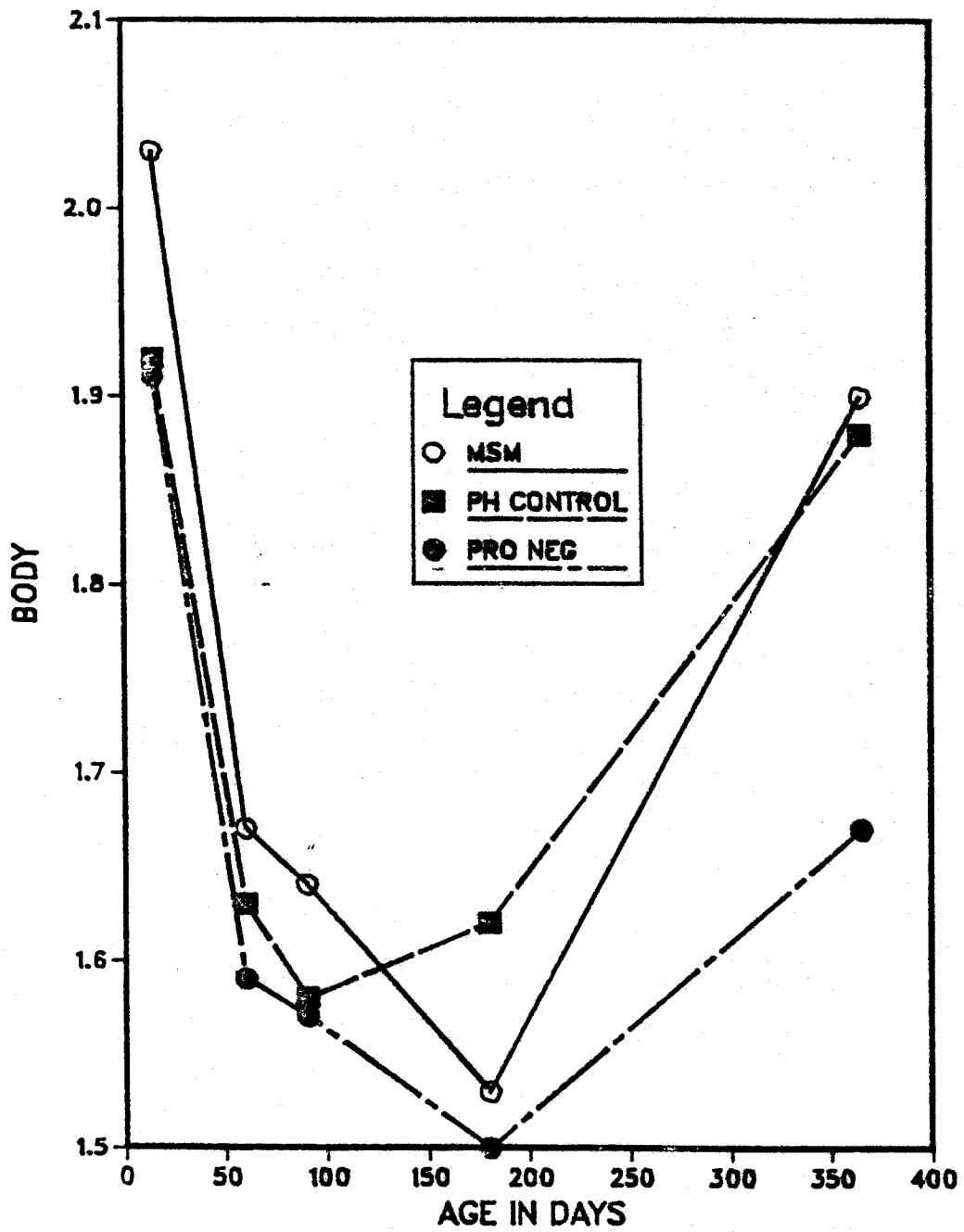
GRADING GUIDE:  
for  
Flavor,  
Body,  
Texture, and  
Total

1	2	3	4	5	6
Superior	Excellent	Satisfactory	Objectionable	very objectionable	unsaleable

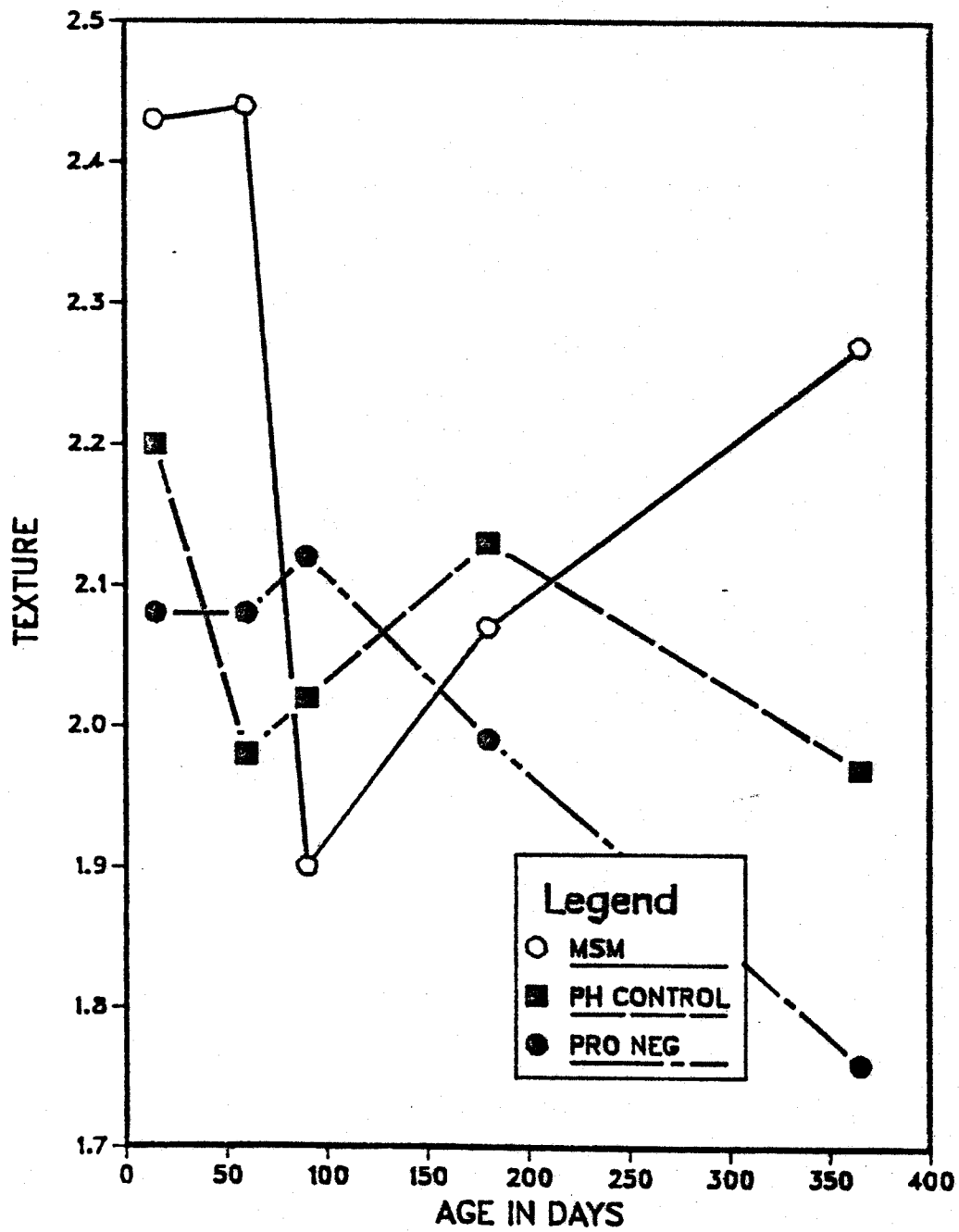
## CHEESE FLAVOR



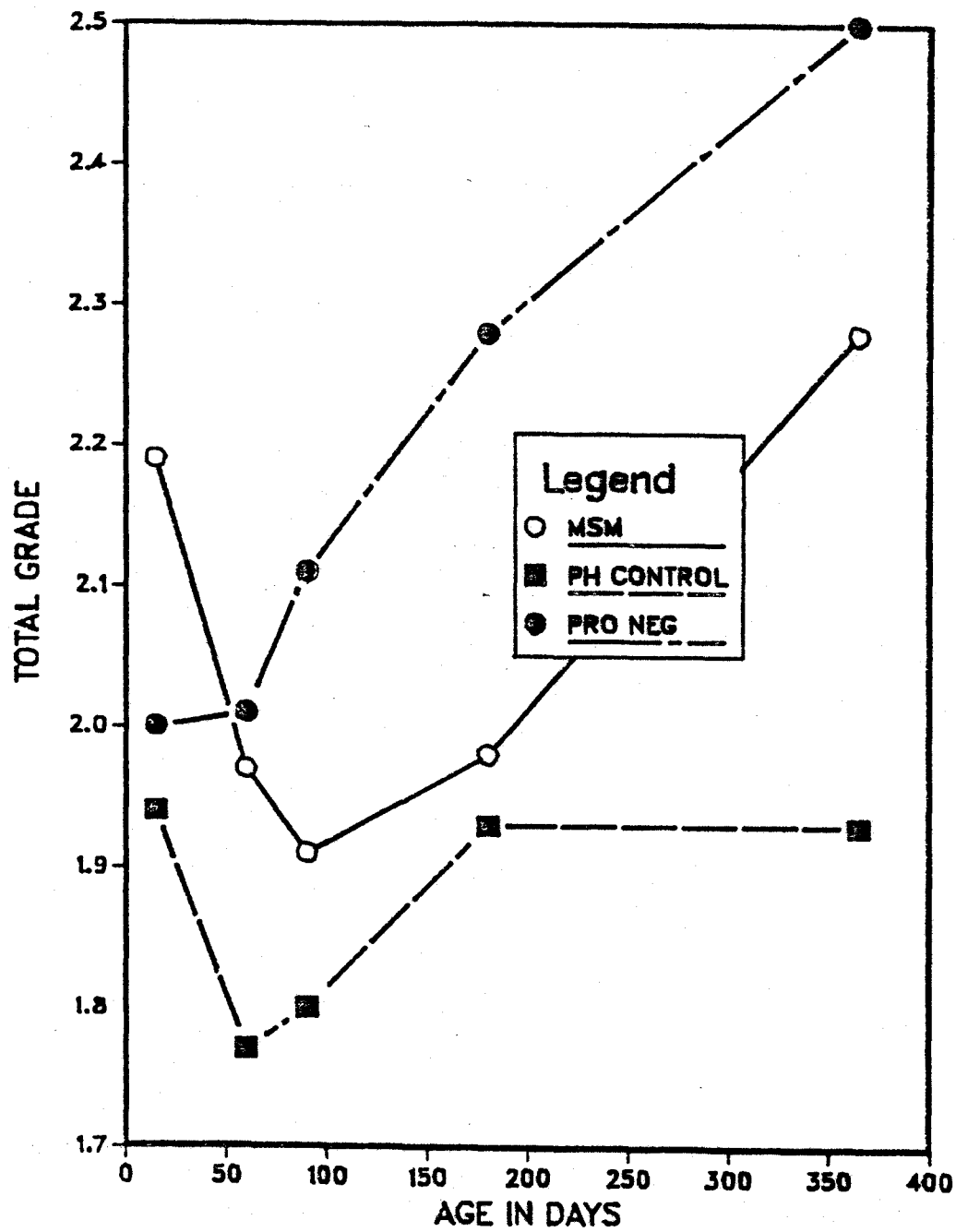
# CHEESE BODY



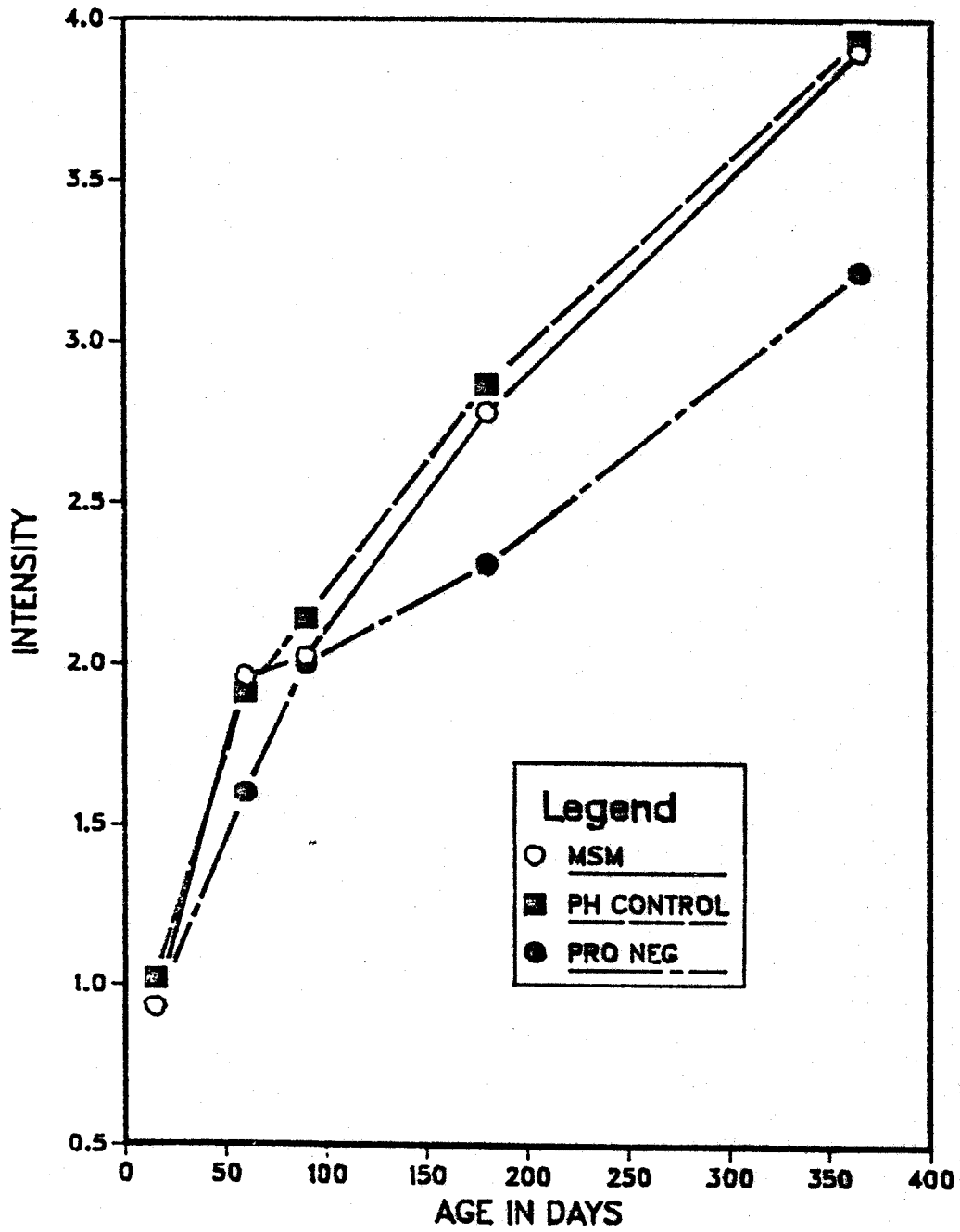
## CHEESE TEXTURE



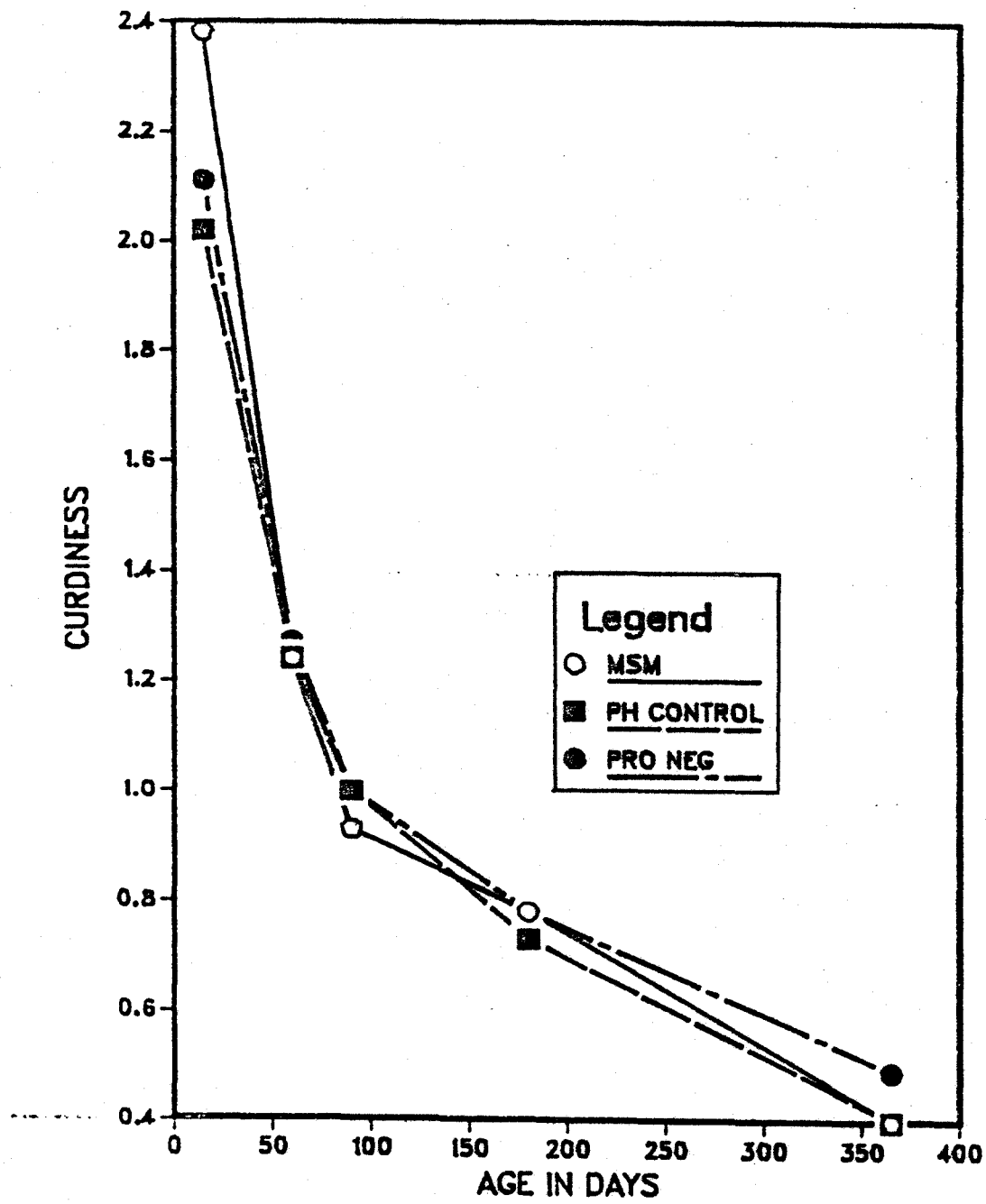
## TOTAL CHEESE GRADE



## CHEESE FLAVOR INTENSITY

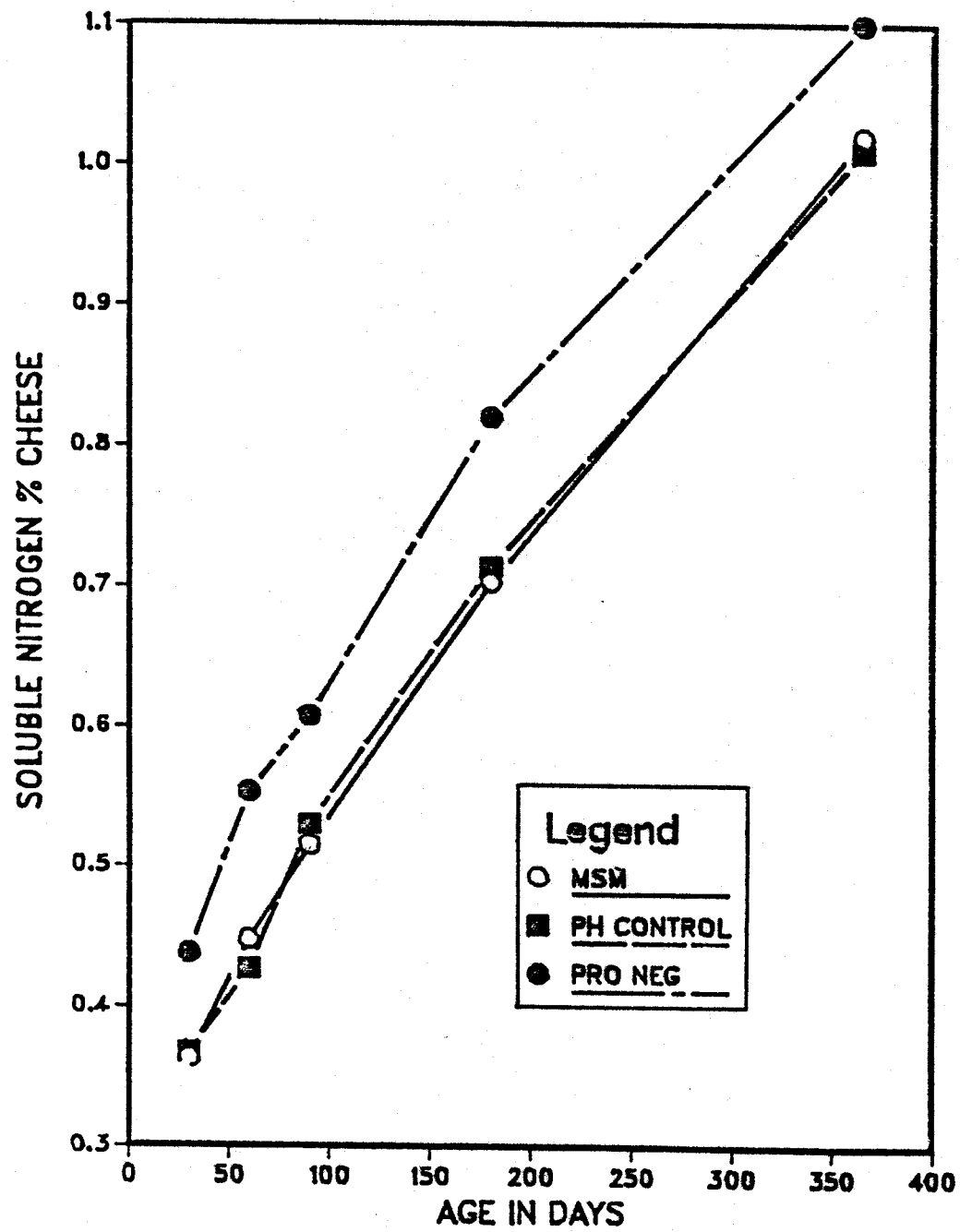


# CURDINESS





## SOLUBLE NITROGEN % CHEESE



Estimation of Milk Protein Concentrations  
by Amino Acid Analysis

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ABSTRACT

A rapid method for estimating concentrations of milk proteins is described. Known amino acid compositions of the proteins are used as coefficients in a series of simultaneous equations. Amino acid concentrations in milk samples can then be used to estimate the amounts of each protein. The method could be used for finding concentrations in any mixture of proteins for which the amino acid composition is known.

## INTRODUCTION

Quantitative separation of proteins in milk is of interest to many researchers. Cheese makers need to know relative amounts of casein and whey proteins in milk. Since whey is often discarded in the cheese industry, new applications for whey utilization are in constant demand. In many cases where whey is an allowed ingredient in a product, standards limit the amount of added whey. Methods for quantitative determination of casein and whey proteins in milk involve precipitation of casein, followed by Kjeldahl nitrogen analysis of both precipitate and whey (2). Further quantitative separation of milk protein into  $\alpha$ s1,  $\alpha$ s2,  $\kappa$  and  $\beta$  caseins,  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin, serum albumin, and immunoglobulins would be a time consuming process. Protein fractions would first be separated by ion exchange or hydroxyapatite chromatography, followed by identification of specific proteins by electrophoresis and quantifying the results with a protein assay on each fraction (1, 8, 13). With this method, highly skilled personnel require days to obtain results. It is a lengthy and costly process.

Whether measuring casein and whey protein or specific proteins such as  $\alpha$ s1,  $\alpha$ s2,  $\kappa$ , and  $\beta$  caseins, the need exists for an accurate and rapid detection method. It would be beneficial to have a rapid method for the detection of whey in powdered milk to aid in regulation of that industry. Since goat milk usually is sold at a higher price than cow milk, the need exists for an efficient method of determining cow milk in goat milk. Not only would it be unethical to sell an adulterated product at the higher

price but problems also exist in the case of individuals allergic to cow milk. Quantitative determination of protein fractions could be used to detect cow milk in goat milk, since goat caseins contain much less  $\alpha$ s casein than bovine casein (18). Amino acid sequence of milk proteins vary between species and these differences could be used to investigate purity of a particular milk type. A method has been described for detecting cow milk in goat milk using polyacrylamide gel electrophoresis (9). The method we propose in this paper should be more rapid and as sensitive for showing the presence of bovine milk in goat milk.

Amino acid composition of each protein in milk is unique and consistent. It is possible that quantitative fractionation of major milk proteins can be determined if amino acid composition of each protein in the mixture is known and amino acid concentration of the mixture measured.

## METHODS

A series of model protein mixtures were formulated with the protein composition of milk or cheese alone or with added protein. Data for milk protein distribution were mean values of that reported by Swaisgood (19). Protein concentrations for a simulated cheese sample were determined for a cheddar cheese, assuming it to contain 22.64% casein and 0.47% whey protein, and that 100 kg milk yields 10.6 kg cheese (21). The method used to calculate amino acid composition of a hypothetical milk sample is demonstrated in Table 1. Given the amino acid profile of each protein and the concentration of the proteins in the milk, expected amino acid concentrations were calculated. Values under the column labeled "Total" are the simulated amino acid analysis values for the example given. Once calculated, these values were used to solve for the various protein fractions. Both actual and estimated protein concentrations are listed in Table 2.

The most common genetic variants of each protein type were selected for the model. In the case of  $\kappa$  casein A and B,  $\beta$ -lactoglobulin A and B, and  $\beta$  casein A1 and A2, a weighted average of the amino acid composition of the variants was used. The average was based on gene frequencies reported for Holstein-Friesian cattle in the U.S. (12).

Once eight proteins were selected, eight amino acids had to be chosen since the method of solution is designed for a square matrix. Because of potential measurement problems, PRO, SER, THR, CYS, MET, TYR, AND TRP were removed from the model. Proline is difficult to measure by many HPLC methods using o-phthaldehyde (see review by Thomas (20)) and the other amino acids may be lost

during hydrolysis (4). ASN and GLN were also eliminated because data were not available for immunoglobulins and serum albumin. Of the remaining amino acids, eight were selected based on their greatest average differences between proteins.

A program for solving simultaneous equations (3) was adapted for use with a Tektronics model 4052 desk top computer. For the example presented in this paper with eight proteins, amino acid compositions of  $\alpha$ s1 casein,  $\alpha$ s2 casein, Kcasein, Bcasein,  $\beta$ -lactoglobulin, and  $\alpha$ -lactalbumin were calculated in g amino acid/ 100 g protein, based on actual amino acid sequences (5-7, 11, 14, 15, 17). Data for immunoglobulins and blood serum albumin were obtained from amino acid analysis (10). Amino acid compositions of the proteins were used as coefficients in a series of simultaneous equations.

Milk samples would first be hydrolyzed in a sealed tube at  $110 \pm 10^\circ\text{C}$  in 6 N HCl for 24 h. (16). Hydrolysates are filtered and then injected into the amino acid analyzer. Amino acid concentrations in the milk sample are determined and then used to solve for concentration of each individual protein. Using the described method, milk protein fractions in an actual sample could be estimated from a single amino acid analysis.

## RESULTS AND DISCUSSION

Using the selection methods described earlier, proteins included in the model were  $\alpha$ -s1 casein B,  $\alpha$ -s2 casein A,  $\kappa$ -casein A and B,  $\beta$ -casein A1 and A2,  $\beta$ -lactoglobulin A and B,  $\alpha$ -lactalbumin B, serum albumin, and immunoglobulins. Amino acids used were ASP, GLU, GLY, ALA, VAL, ILE, LEU, and LYS.

Amino acid compositions of six hypothetical protein mixtures were calculated and used as examples to solve for protein concentration. In our hypothetical situation, predicted protein concentrations were very close to expected values (see Table 2.). In all cases, expected and calculated values were within  $\pm 0.02$  g/100 g or g/100 ml sample (see differences in Table 2). These differences are probably due to rounding, and would not pose as a problem in the method.

Further investigation could include a comparison of classical methods of protein quantification with prediction from amino acid analysis. If a sample contained significant amounts of free amino acids, this is a potential source of error in predicting protein concentration. However, free amino acids in a product such as milk would pose no problem if levels remained low and consistent. It would be impossible to determine the extent of proteolysis that had occurred because the method will make predictions based upon the original protein composition.

This technique could be used to quantify casein and whey protein in dairy products. It could also be useful for detection of added whey protein in products which have legal guidelines for the amount of whey allowed. Milk proteins of other species,

including humans, could be studied quantitatively using this method. Studies of human milk fractions could aid in the development of appropriate supplemental infant formulas. The method has potential as a rapid and reliable screening technique for detecting cow milk in goat milk products. Additionally, other mixtures of proteins with known amino acid structures, could be quantitatively determined. Use of the technique could be applied to other areas of food science, nutrition, and clinical chemistry.

A complete quantitative determination of the protein fractions in milk could be obtained in about 26 h. Most of the analysis time would be due to time required for hydrolysis and would not be labor intensive. The method has potential as an important tool for all researchers interested in measuring protein fractions in milk.



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Table 1. Method used to estimate amino acid composition (g/100 ml) of hypothetical milk sample using actual protein concentrations<sup>a</sup> (g/100 ml) and known amino acid composition<sup>b</sup> (g/100 g) of each protein.

	as1 casein	as2 casein	k casein	b casein	b-lact glob	a-lact alb	serum alb	imm glob	Total
Milk	1.35	0.35	0.35	1.00	0.30	0.125	0.025	0.080	
Asp-prot	3.95	2.11	3.322	2.218	7.628	8.451	10.9	9.40	
Asp-milk <sup>c</sup>	0.0533	0.0074	0.0116	0.0222	0.0229	0.0106	0.0027	0.0075	0.1382
Glu-prot	14.95	14.58	9.286	11.03	12.85	8.304	16.5	12.3	
Glu-milk	0.2018	0.0510	0.0325	0.1103	0.0386	0.0104	0.0041	0.0098	0.4585
Gly-prot	2.861	0.5951	0.7897	1.564	1.435	3.178	1.8	5.2	
Gly-milk	0.0386	0.0021	0.0028	0.0156	0.0043	0.0040	0.0005	0.0042	0.0721
Ala-prot	3.396	2.825	6.912	1.856	7.052	1.886	6.3	4.8	
Ala-milk	0.0458	0.0099	0.0242	0.0186	0.0212	0.0024	0.0016	0.0038	0.1275
Val-prot	5.457	6.501	6.778	9.274	6.075	4.959	5.9	9.6	
Val-milk	0.0737	0.0228	0.0237	0.0927	0.0182	0.0062	0.0015	0.0077	0.2465
Ile-prot	6.111	5.72	8.797	5.466	7.161	7.404	2.6	3.0	
Ile-milk	0.0825	0.0200	0.0308	0.0547	0.0215	0.0093	0.0007	0.0024	0.2219
Leu-prot	9.444	6.76	5.52	12.02	15.75	12.03	12.3	9.6	
Leu-milk	0.1275	0.0237	0.0193	0.1202	0.0473	0.0150	0.0031	0.0077	0.3638
Lys-prot	8.668	13.91	6.92	6.70	11.97	12.38	12.8	6.8	
Lys-milk	0.1170	0.0487	0.0242	0.0670	0.0359	0.0155	0.0032	0.0054	0.3169

<sup>a</sup>Reference 19

<sup>b</sup>References 5-7, 10, 11, 14, 15, 17

<sup>c</sup>Example:

1.35 g as1 casein/100 ml milk \* 3.95 g ASP/100 g as1 casein = 0.0533 g ASP/100 ml milk

Table 2. Actual protein concentration<sup>a</sup>, concentration estimated by method described in this paper, and differences between the two values.

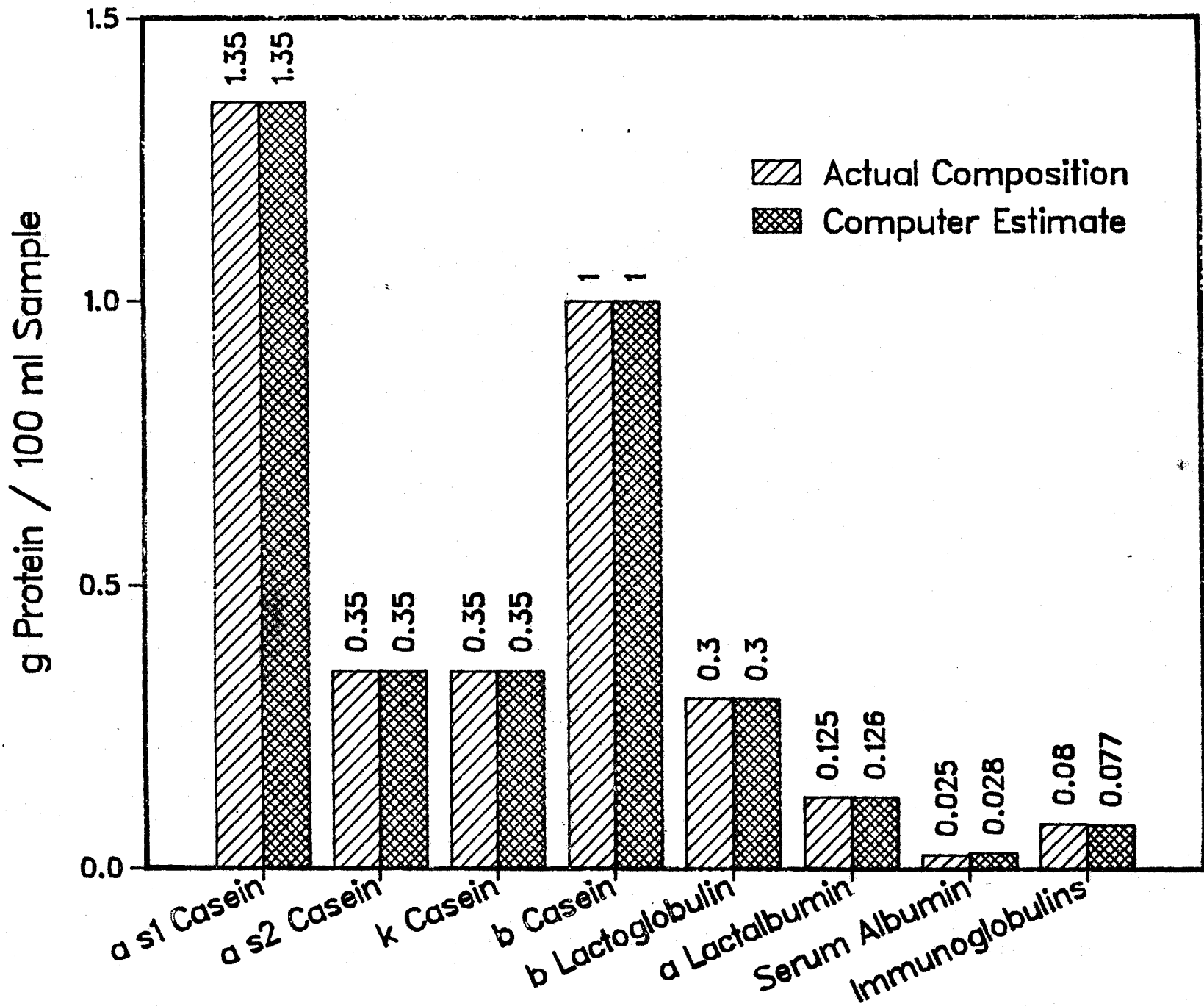
Sample		as1 casein	as2 casein	k casein	b casein	b-lacto- globulin	a-lact- albumin	serum albumin	imm glob
Milk <sup>b</sup>	act	1.35	0.35	0.35	1.00	0.30	0.125	0.025	0.080
	est	1.35	0.35	0.35	1.00	0.30	0.126	0.028	0.077
	diff	0.00	0.00	0.00	0.00	0.00	0.001	0.003	0.003
Milk + 5% Prot	act	1.42	0.368	0.368	1.05	0.315	0.131	0.026	0.084
	est	1.42	0.367	0.367	1.05	0.315	0.131	0.027	0.084
	diff	0.00	0.001	0.001	0.00	0.00	0.00	0.001	0.00
Milk + 5% WP	act	1.35	0.35	0.35	1.00	0.315	0.131	0.026	0.084
	est	1.35	0.35	0.35	1.00	0.312	0.131	0.030	0.082
	diff	0.00	0.00	0.00	0.00	0.003	0.000	0.004	0.002
Cheese <sup>c</sup>	act	10.02	2.60	2.60	7.42	0.266	0.111	0.022	0.071
	est	10.00	2.58	2.60	7.43	0.255	0.105	0.038	0.060
	diff	0.02	0.02	0.00	0.01	0.011	0.006	0.016	0.011
Cheese + 5% casein	act	10.52	2.73	2.73	7.80	0.279	0.116	0.023	0.074
	est	10.50	2.73	2.73	7.80	0.274	0.120	0.026	0.074
	diff	0.02	0.00	0.00	0.00	0.005	0.004	0.003	0.000
Cheese + All WP in Milk	act	10.02	2.60	2.60	7.42	3.74	1.56	0.312	0.996
	est	10.00	2.60	2.59	7.43	3.74	1.55	0.309	0.997
	diff	0.02	0.00	0.01	0.01	0.00	0.01	0.003	0.001

<sup>a</sup>Data are from Swaisgood(19).

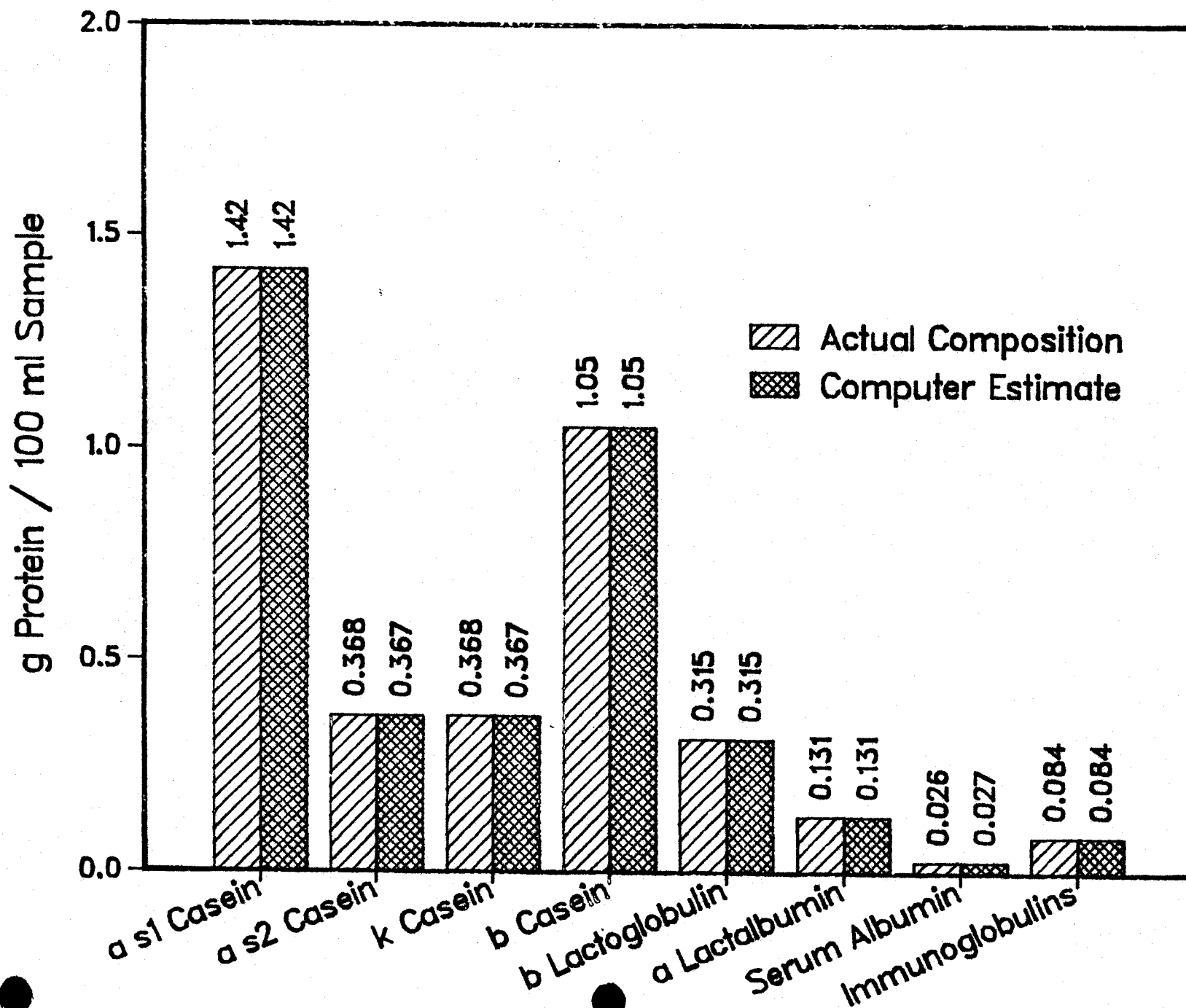
<sup>b</sup>Concentration in g/100 ml.

<sup>c</sup>Concentration in g/100 g.

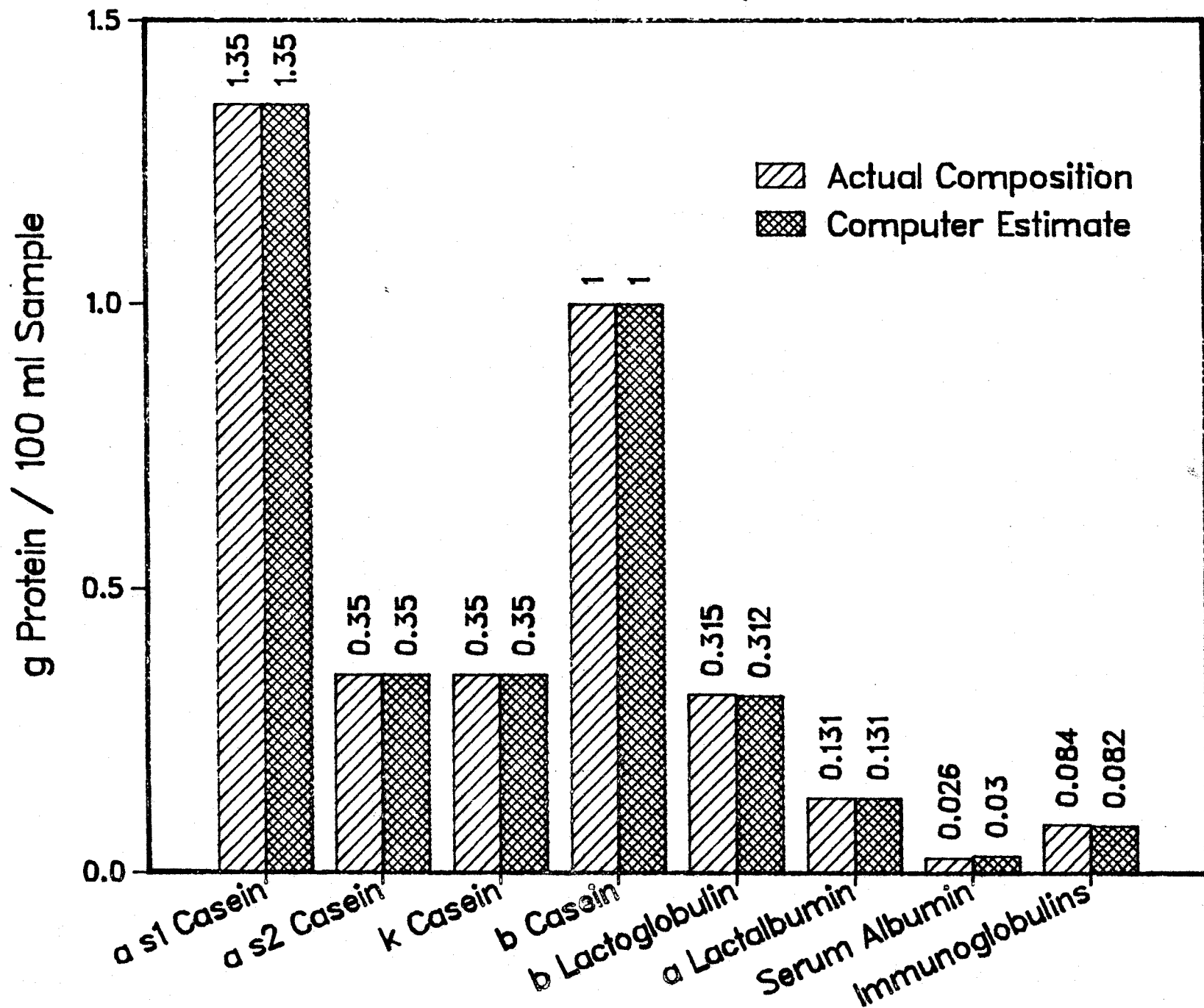
# Milk



# Milk + 5 % Milk Protein

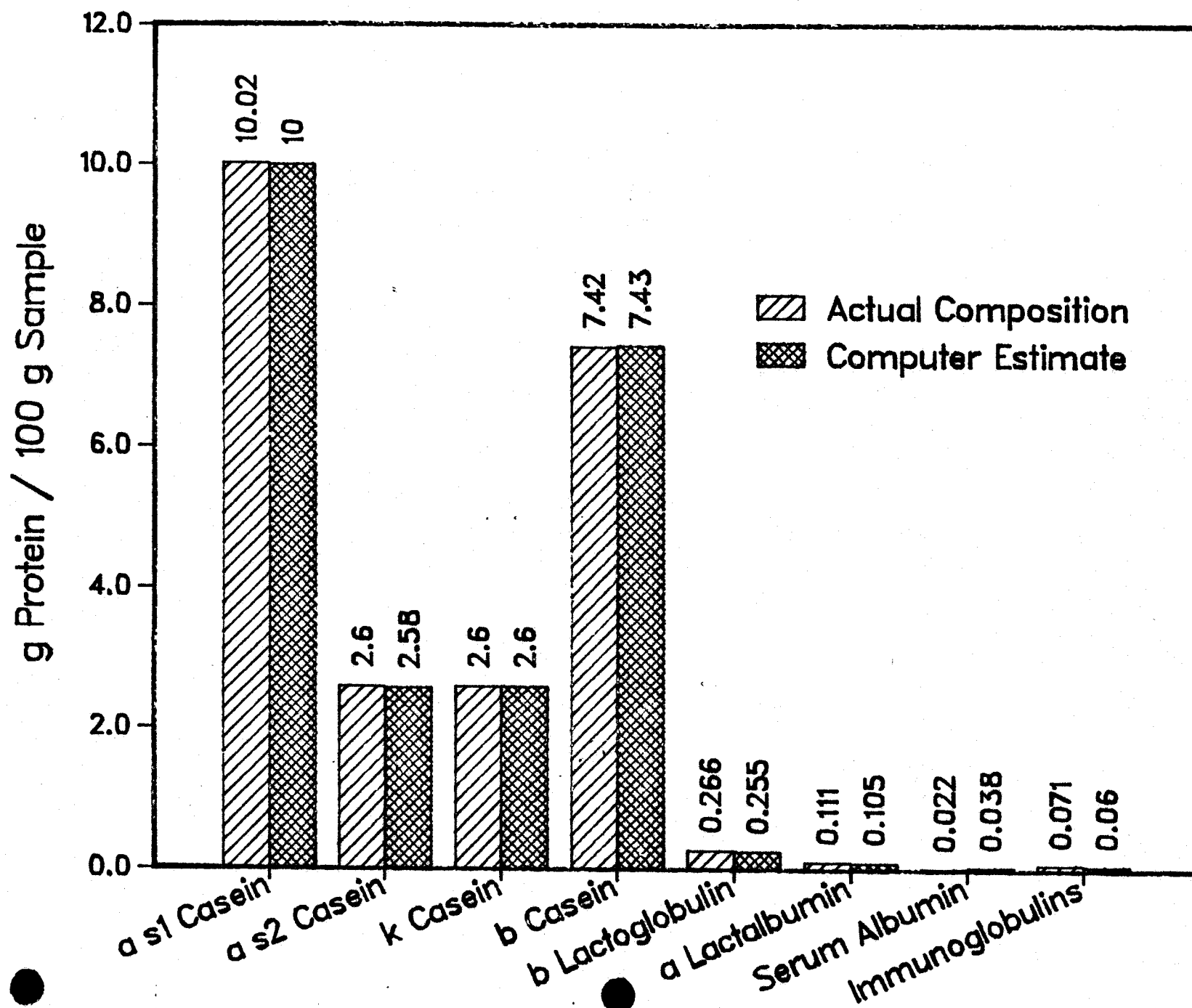


# Milk + 5 % Whey Protein

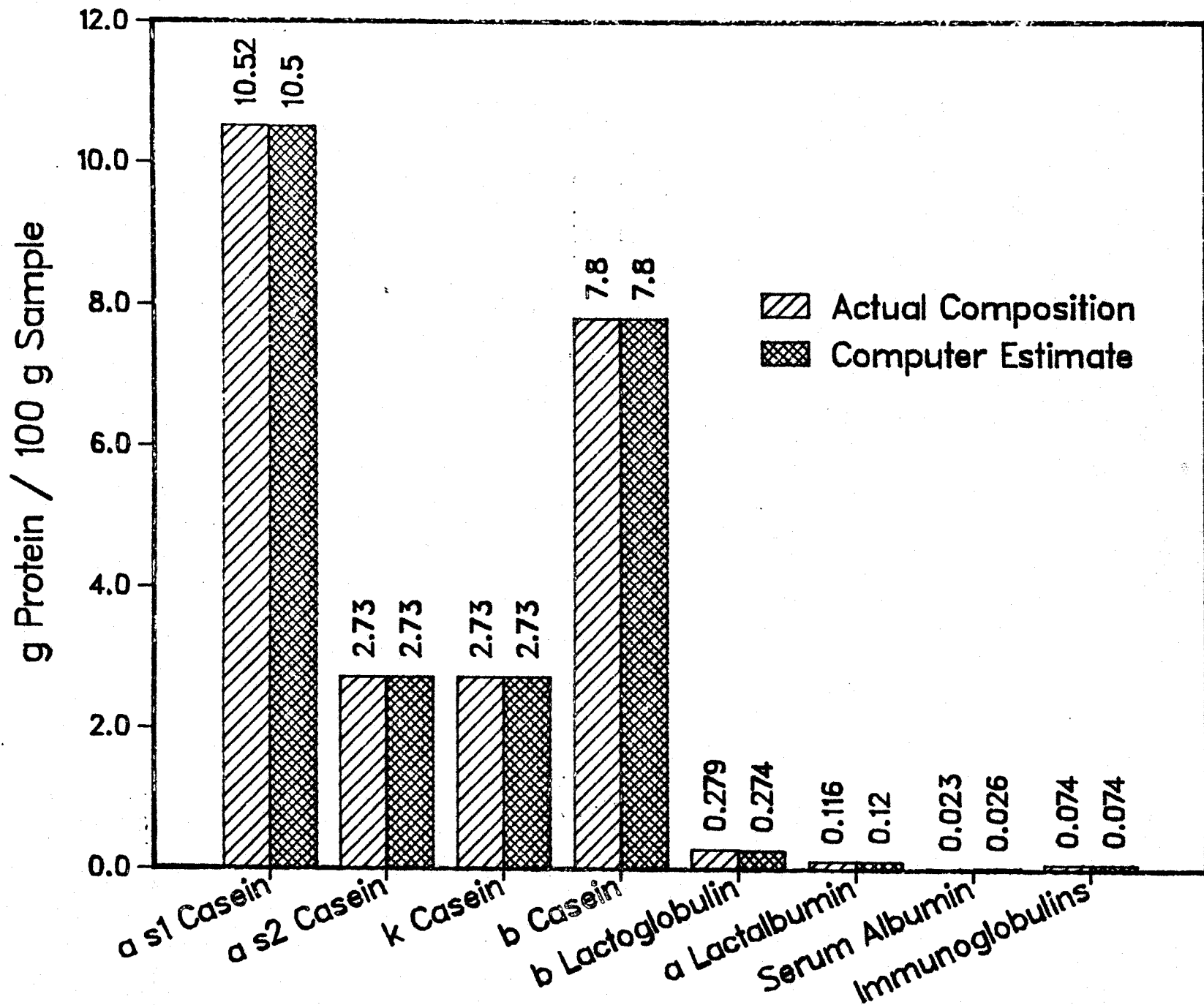




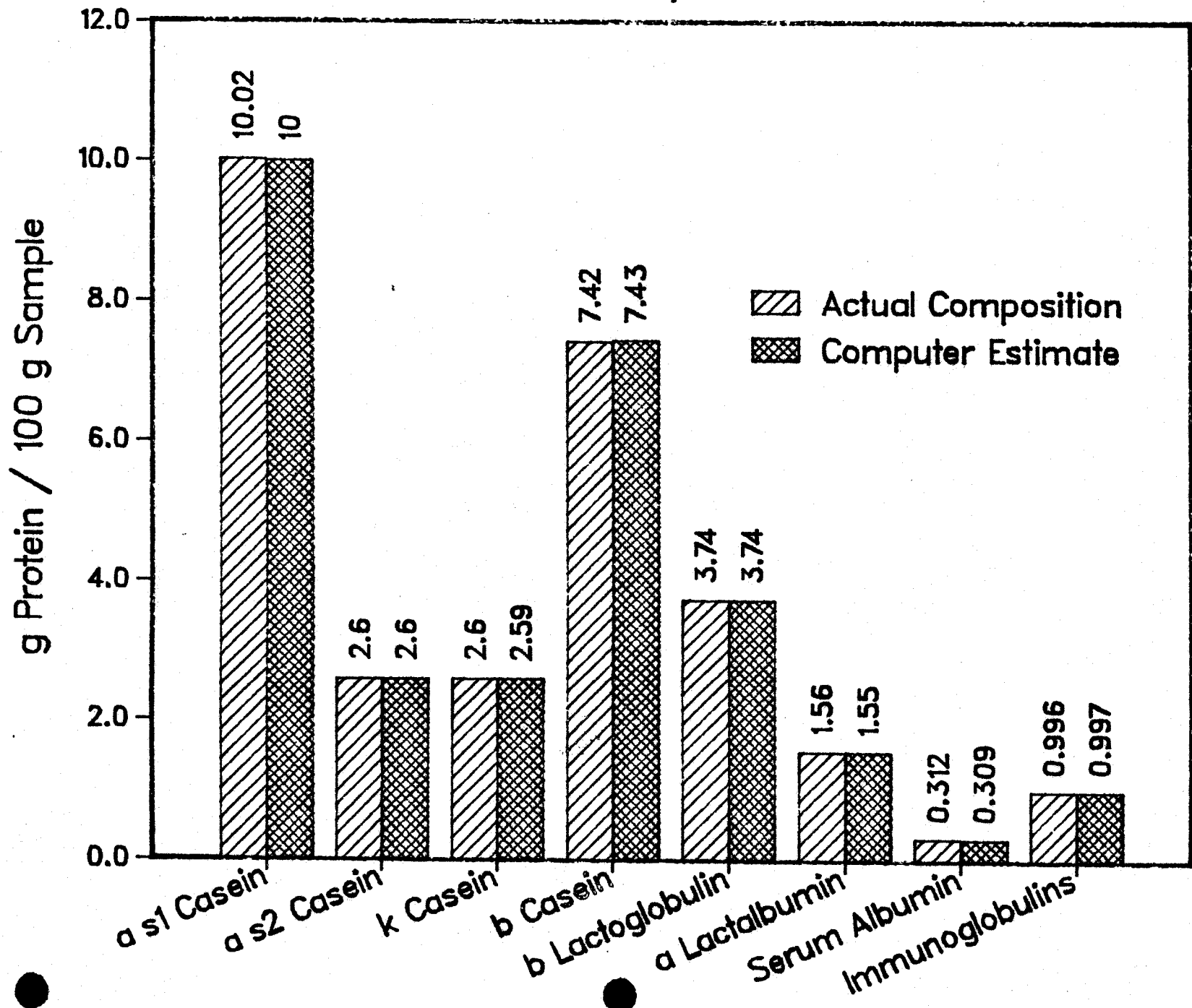
# Cheese



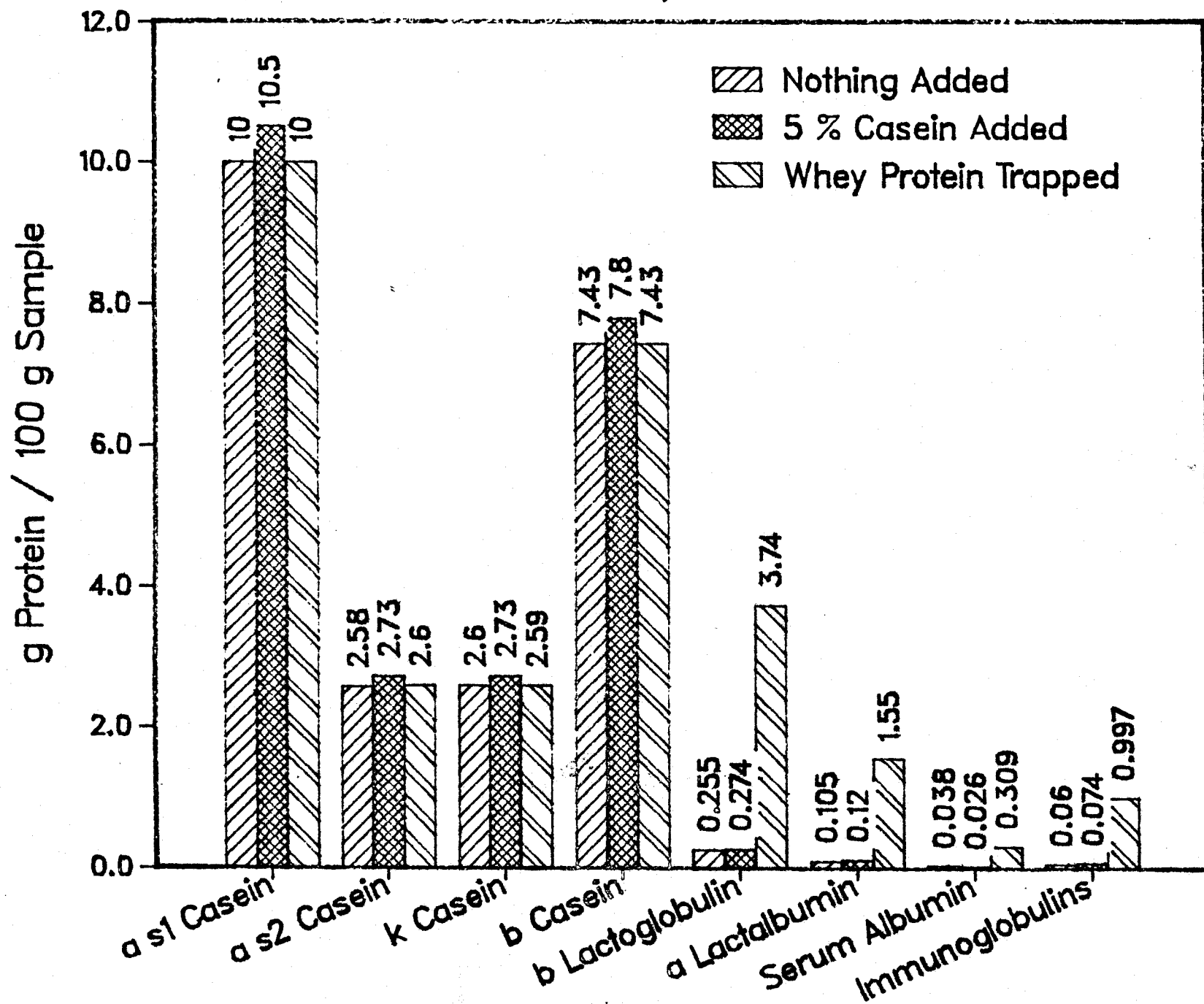
# Cheese + 5 % Casein



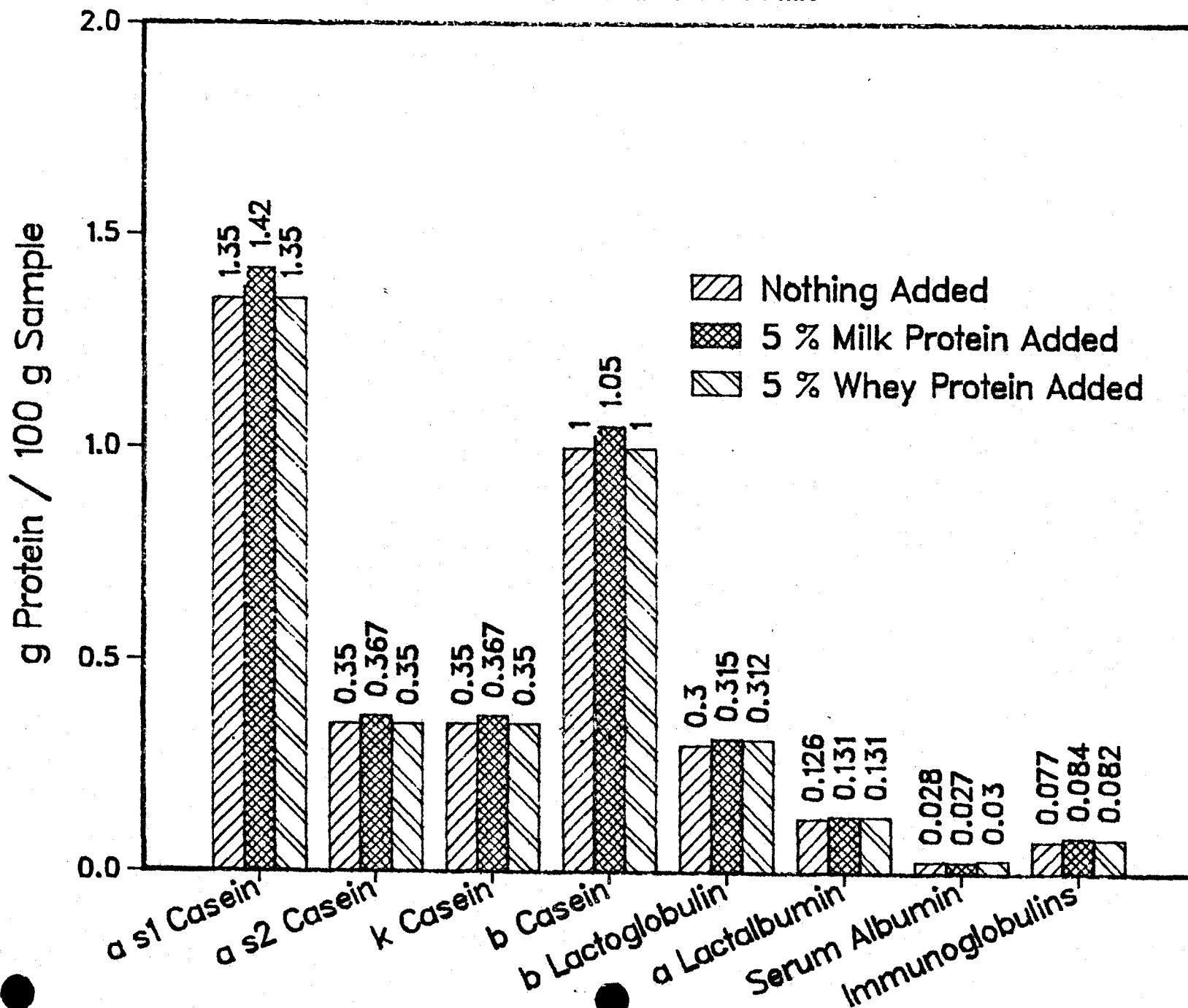
## Cheese with Whey Protein Included



# Computer Estimates of Composition From Amino Acid Analysis of Various Cheeses



# Computer Estimates of Composition From Amino Acid Analysis of Milk and Adulterated Milk



EXERCISE AND CHEESE -SUPPLEMENTED DIET EFFECTS  
ON LEVELS OF CHOLESTEROL AND LIPOPROTEIN FRACTIONS  
IN HUMAN SUBJECTS

by  
Kathe Gabel-Lind, M.S., R.D.

Public interest in cholesterol is high. Popular magazines and the news media have related dairy products with significant cholesterol levels. Cheese, in particular, has been suggested as having a high cholesterol content, even though it compares favorably with other food products perceived as low cholesterol. Food industries whose products contain cholesterol have launched advertising campaigns to fight this "negative" publicity.

The dairy industry could combat the prevalent wave of cholesterol hysteria with a similar type of promotion or encourage research pertaining to the hypocholesteremic or normocholesteremic effect of dairy products on serum lipids and lipoproteins.

The main objective for the proposed study is to determine the effect of exercise and ingestion of cheese on serum lipids and lipoprotein fractions in human subjects. Healthy, normal weight students from Utah State University will be used to complete the project. Cholesterol, tri-glyceride, lipoprotein and calcium levels will be assessed before, during, and after the study.

The story about cholesterol is an old one. It started as early as 1769 when a French chemist crystallized a gallstone and prepared the first pure cholesterol. Later in 1816, another French chemist named cholesterol using the Greek words -"chole" and "steros".

Cholesterol again appeared in the literature in the 1950's when studies to find the causes of heart disease were initiated. A number of intervention trials were conducted in the 1960's and 1970's, in which attempts were made to lower serum cholesterol levels in a defined population. In the 1980's, more intervention studies have emerged in which cholesterol levels have been studied. The most recent being the Type II Coronary Intervention study conducted by the National Heart, Lung, and Blood Institute and the Lipid Research Clinic-Coronary Primary Prevention Trial (2).

People often associate cholesterol with negative conditions, ie., Coronary Heart Disease (CHD), heart attack, stroke, and even death. However, in recent years, "good and bad" cholesterol has been differentiated. In 1975, Miller and Miller (3) indicated that high density lipoproteins (HDL) have an inverse relationship to the risk of CHD. Of all the lipoproteins ie., chylomicrons, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins, HDL seems to be protective against coronary heart disease.

Elevated serum cholesterol is regarded as a risk factor in CHD. Dietary cholesterol and fatty acids have been reported to influence the level of cholesterol in the blood. Key's formula (4) predicts the change in serum cholesterol with alterations of dietary cholesterol and fatty acids in the diet.

Over the past twenty-five years, numerous diet modifications have been suggested by physicians, associations, and at least eighteen scientific committees to decrease CHD risk. As suggested by the Inter-Society commission for Heart Disease Resources, the recommended diet modifications

basically reflect a calorie-controlled, moderately low cholesterol, and moderately low saturated fat diet (5). To adhere to this type of diet, one of the changes suggested includes the "replacement of fat-rich meats, dairy products, and baked goods with lower fat types"(6).

Milk and dairy foods have been considered hypercholesterolemic. However, in a review by Richardson (7), Maasai tribesmen in Africa are reported to have low serum cholesterol levels and a low incidence of CHD, while consuming four to five liters of fermented whole milk and substantial amounts of meat per day. An experiment by Bazzano and Olson (8) showed that consumption of whole milk as the major source of nutrients plus a limited amount of carbohydrate is no more hypercholesteremic than the usual American diet. Yogurt and calcium supplementation has been shown to alter cholesterol concentrations more in females than males. In the same study, yogurt, but not calcium supplementation, resulted in a significant decrease ( $p < .001$ ) in fasting cholesterol levels in females (9).

More research is necessary to substantiate the hypocholesteremic effect, if any, of various dairy products. Individuals may be changing their dietary habits needlessly in the direction of less dairy products, without sound scientific basis. Because milk is a major source of protein, calcium, magnesium and riboflavin, dairy foods contribute significantly to the American diet. The inclusion of dairy products is especially important for the possible prevention of osteoporosis, a bone dissolution disease. The emphasis on maintenance of physical activity and consumption of adequate calcium throughout one's lifetime is increasing for the prevention of the disease.

Physical activity is also viewed as an influence upon serum levels of



lipids and lipoproteins. Previously sedentary men significantly increased high density lipoprotein fractions, a protective factor against CHD, and reduced triglyceride and low density lipoprotein levels with a program of mild to moderate exercise (10). Shorey, Sewell, and O'Brien (11) studied the effect of diet and exercise on serum lipids. Results were suggestive of a beneficial effect of exercise in addition to the diet in accelerating or maintaining reductions in serum lipids. Most research suggests that increased exercise levels lead to lower blood concentrations of triglycerides, VLDL, and LDL. HDL levels tend to increase with exercise.

To reiterate, the main objective for this study is to determine the effect of exercise and ingestion of cheese on serum lipids and lipoprotein and lipoprotein fractions in human subjects.

#### METHODS

Because the cheese market is predicted to grow 11% (12) during the 1980's, the ease of handling, and storage, and no previous testing for a hypocholesteremic effect, cheese has been chosen as the experimental dairy product for the study.

Female and male human subjects, 18 to 22 years old, will be randomly chosen from Utah State University living quarters. Fifty normal weight and disease free individuals will be requested to consume pre-determined food intakes to maintain their weight and to control diet composition. Food intake will be recorded by dietetic assistants and researcher everyday and every meal during the project. Eating at establishments other than the campus cafeteria will be discouraged but if they do, diet records will be requested.

Cheddar cheese in four ounce portions will be supplemented daily to

the meals consumed by participants during two 18 day periods. Nutrient intake will be analyzed with the use of the food composition data banks provided by the Department of Nutrition and Food Sciences at USU.

Concerning the exercise component of the study, the participants will be randomly placed into two groups of 25 subjects each. After being matched by sex, age, height/weight index, and blood lipid level, the groups will be designated as "sedentary" and "exercise".

The "sedentary" group will be requested not to engage in any regular conditioning program or sports program during the test period. Enrollment in and attendance to a conditioning class sponsored by the Health, Physical Education and Recreation Department will be required of the exercise group during the same time period. A physical education graduate assistant and researcher will supervise the conditioning of the exercise group five days a week for a forty minute period.

Blood lipids and lipoprotein fractions will be assessed initially and every two and a half weeks following the beginning of the study. Blood calcium levels will be assessed at the same as the lipids. After collection of all data, changes in blood parameters will be determined and results analyzed.

#### EXPECTED OUTCOME

By controlling the composition of diets consumed with supplemented dairy products and controlling all possible factors affecting serum lipids, the question of whether or not cheese has a hypo- or normocholesteremic effect may be clarified. The addition of exercise to the diet regimen may present a synergistic effect which in many ways could improve one's health. Improvements could include a decrease in total cholesterol, an increased

HDL/LDL ratio, improvement in physical fitness, an increase in positive calcium balance, and possibly assist in the prevention of CHD and/or osteoporosis.

Depending on the outcome of the immediate investigation, future extension of this project could include the study of other dairy products of different age groups.

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Proposed Budget

17 DRAB members	\$85,000
Utah Agricultural Experiment Station	<u>20,000</u>
TOTAL	\$105,000

10 graduate student stipends including 1 research technician	68,000
Laboratory assistance	3,600
Milk and supplies	9,000
Equipment	20,000
Travel	<u>4,400</u>
TOTAL	\$105,000

## Projects for 1984-85

1. Production of cottage cheese from U.F. skim milk.
2. Role of milk clotting enzymes in cheese curing. *Sign of milk Coag Curves shape on milk quality.*
3. Use of proteinase negative cultures in buttermilk, sour cream, cottage cheese and Cheddar cheese.
4. Development of gene transfer systems and recombinant DNA techniques to improve Streptococcus cremoris starter strains.
5. Improving properties of natural and process cheese made from U.F. whole milk.
6. Estimation of milk protein concentration by amino acid analysis.
7. Effect of exercise and cheese supplemented diets on levels of cholesterol and lipoprotein fractions in human subjects.
8. Utilization of lactose hydrolyzed syrups from whey and milk permeates.